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COMPARISON OF SHOOT APEX AND LEAF DEVELOPMENT AND STRUCTURE IN DIPLOID AND TETRAPLOID MAIZE[1]

By L. F. RANDOLPH, *cytologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture,* and *professor of botany, New York State College of Agriculture;* ERNST C. ABBE, *associate professor of botany, University of Minnesota;* and JOHN EINSET, *assistant in plant breeding, New York State College of Agriculture*

INTRODUCTION

It is well known that autopolyploids, originating by direct doubling of the chromosome complement, often are morphologically different from the parental forms. The term "gigas," which has been associated with the autotetraploid state since the early work on *Oenothera*, carries with it a series of connotations concerning the manner in which the form of the tetraploid organism differs from that of its diploid relatives. But as East (*4*)[2] and others have emphasized, not all autopolyploids are true gigas types. Numerous qualitative observations have been made on the morphology of experimentally induced autotetraploids, and the investigations in this field were comprehensively reviewed by Müntzing (*6*) in 1936. In the past few years additional qualitative observations have been made, especially on the mature state, yet relatively few precise quantitative morphological studies of autotetraploids have been made.

It is becoming increasingly apparent that the effects of direct chromosome doubling are extremely diverse, especially when attention is focused on specific or individual characteristics. These effects may differ not only in different species but also in different genetic entities within the species. We are here dealing with inherited form differences which, ostensibly at least, cannot be attributed to gene differences of the ordinary sort. Obviously, the change from the diploid to the tetraploid state involves a doubling of the number of genes, but there is presumably no change in the kinds or relative proportions of the genes. It is extremely important to know why it is that different genotypes respond differently to chromosome doubling. In certain instances it may be due to cumulative versus noncumulative gene action, as suggested by Randolph and Hand (*10*) in connection with analyses of the carotenoid content of diploid and tetraploid

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[2] Italic numbers in parentheses refer to Literature Cited, p. 76.

maize; but little is known about the genetics of morphological differences induced by chromosome doubling.

With respect to these morphological differences, it is of primary importance to determine just what their essential nature is, both in the developmental history of the plant and at maturity. In attempting critical morphological comparisons of comparable diploids and tetraploids, the quantitative approach is of basic significance since it permits more accurate characterization of the effect of doubling the number rather than modifying the quality of the genes. Accordingly, quantitative studies of both developmental and mature manifestations of autotetraploidy in maize were undertaken with due regard to possible genetic implications.

The leaf was selected as perhaps the most suitable organ of the plant for such comparative morphological analyses, as it has a relatively simple form whose external dimensions and internal cellular constituents can be measured with reasonable accuracy at all stages of development. In diploid maize the increased width of successively younger leaves, from leaf 6 through leaf 12, is definitely correlated with an increase in the size of the shoot apex (*2*). With this evidence at hand, it seemed especially desirable to investigate the size relations of cells, tissues, and organs in the shoot apex and the leaf during ontogeny and at maturity in comparable strains of diploid and tetraploid maize.

MATERIAL AND METHODS

The diploid and tetraploid strains used in the ontogenetic studies had a common origin. Tetraploidy was induced by heat treatment (*7*) in an F_1 hybrid between inbred lines of Webbers Dent and Illinois A, pollinated by an inbred line of Luces Favorite. The resultant tetraploid plants, several in number, were then mass-pollinated for 2 generations to provide adequate material for the investigation. Diploid sister plants of the original induced tetraploids were similarly mass-pollinated for 2 generations to produce a diploid strain as nearly comparable to the tetraploid as possible. These were the strains utilized by Randolph and Hand (*10*) in a study of the relation between carotenoid content and the number of genes per cell in diploid and tetraploid maize. To insure the selection of comparable diploid and tetraploid material from these stocks, a considerable number of ear-row progenies were grown from the diploid and tetraploid strains, from which were selected 4 tetraploid and 4 diploid ears whose progenies were representative of the range of variation in the 2 strains. From each ear a minimum of 120 kernels were planted in 5 replications and grown during the summer of 1938 in a soil-filled bench in the greenhouses of the Botany Department, Cornell University. Similar plantings were also made in the experimental gardens of the Department of Plant Breeding, Cornell University, to provide material for a comparison of mature-leaf dimensions in field-grown plants. Additional stocks utilized in the comparisons of mature-leaf dimensions will be described later.

Details concerning collections, dissection techniques, fixing, sectioning, and staining applicable to the following developmental study have been published elsewhere (*2*). The histological studies of the mature leaf described in the second part of this paper involved the use of either frozen microtome sections or hand sections of fresh

material. For these studies hand sections proved eminently satisfactory, not only because they could be prepared expeditiously, but also because a higher degree of accuracy in quantitative histological studies results from a minimum of departure from the living state.

The tenth leaf blade, i. e., the leaf blade located at the tenth node above the coleoptile, was selected as representative of the approximately 16 leaves ordinarily produced by maize varieties of this locality. It was evident, from the relative growth curves obtained in the previous study of leaf development in diploid maize (*2*), that the leaves produced during the period of maximum vegetative activity (leaves 6 through 12) are similar in general growth pattern; but because of the consistent differences between successive leaves, it seemed desirable to select a leaf at one particular position for the present study. Therefore, the tenth leaf (counting the first leaf above the coleoptile as leaf 1) was selected for detailed analysis. This is the leaf that ordinarily subtends the functional ear shoot of the mature plant.

Since each leaf originates as a primordium that develops as a ridge about the base of the shoot apex, an understanding of the developmental relations of the leaf requires a knowledge of the organization of the shoot apex itself.

SHOOT APEX DURING PLASTOCHRON 10

The shoot apex of maize in the seedling stage is an elongate paraboloid of revolution, from the base of which arise the primordia of the leaves in acropetal succession (fig. 1). In the mature kernel the cole-

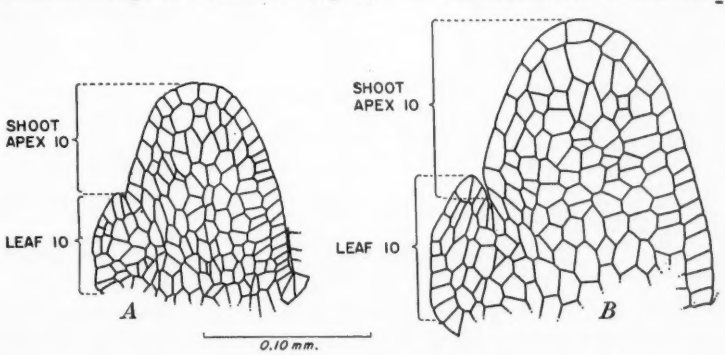

FIGURE 1.—Shoot apex and primordium of leaf 10 in median longitudinal section, showing relative dimensions of cell and organ in (*A*) diploid and (*B*) tetraploid maize. Camera lucida drawings of representative shoot apices.

optile and five seedling leaves already are partly formed, and in the developing seedling and young plant additional primordia develop successively at intervals of 2 to 3 days under optimum growing conditions. The primordium of the tenth leaf is normally being formed in seedlings about 8 to 10 days after planting. The time interval during which the leaf primordium is differentiating from the shoot apex is designated by Askenasy's term "plastochron." When the tenth leaf primordium is differentiating, the shoot apex is said to be in the tenth plastochron. The shoot apex during the tenth plastochron formed the basis for the following observations.

It was readily possible in the seedling stage to dissect out separately the shoot apex, the leaf primordia, and the immature leaves at various stages of development, by means of the technique described by Abbe, Randolph, and Einset (*2*). The entire shoot apex and the young leaves were measured directly to determine their gross dimensions. Thereafter, a certain number of these same shoot apices were embedded in paraffin and sectioned to provide material for detailed analyses of their cellular structure.

The absolute size of the diploid shoot apex during the tenth plastochron differed markedly from that of the tetraploid. The basal diameter of the diploid averaged 130μ as contrasted with an average of 154μ for the tetraploid. In length the diploid averaged 73μ and the tetraploid 94μ (table 1). Thus the tetraploid shoot apex was very significantly larger than the diploid shoot apex.

TABLE 1.—*Size relations of diploid and tetraploid shoot apices during plastochron 10*

Item	Data on shoot apices			N[1]	Method of obtaining measurements
	Diploid	Tetraploid	Ratio ($4n/2n$) on a linear basis		
1. Basal diameter.....μ	[2] 130.0±[3] 3.0	154.0±[3] 3.0	[4] 1.19*	25	Direct measurement.
2. Length.....μ	73 ± 3.0	94 ± 3.0	1.29*	15	Do.
3. Basal circumference.....μ	408	484	1.19	....	Item 1×π.
4. Area in median section.....μ^2	6,300	9,600	$1.24(\sqrt{1.53})$	....	$\frac{2}{3}$ d h.
5. Volume.....μ^3	485,000	875,000	$1.22(\sqrt[3]{1.81})$	....	$\frac{1}{2}$ πr^2 h.
6. Cells in median longitudinal section.	No 60.8± 4.4	No 56.8± 2.7	.93	16	Direct observation.
7. Average cell area.....μ^2	104	169	$1.28(\sqrt{1.63})$	....	Item 4/item 6.
8. Cells in basal diameter.	No 10.1± .4	No 9.9± .4	.98†	16	Direct observation.
9. Average cell diameter.....μ	12.9	15.6	1.21	....	Item 1/item 8.
10. Average cell volume.....μ^3	1,342	2,636	$1.25(\sqrt[3]{1.96})$	....	Item 7×item 9.
11. Nuclear diameter.....μ	7.4± .08	9.3± .08	1.26*	200	Direct measurement.
12. Nuclear volume.....μ^3	212	421	$1.26(\sqrt[3]{1.99})$	....	$\frac{4}{3}$ πr^3.

[1] N=total number of samples.
[2] The slight discrepancy between this value and the corresponding one in Abbe, Randolph, and Einset (*2*) is to be attributed chiefly to a difference in the population sampled.
[3] Standard error.
[4] Student's t was used to determine the significance of the difference between tetraploid and diploid. An asterisk (*) indicates that the odds were greater than 19:1 and that the values are therefore significantly different; a dagger (†) indicates that the values are not significantly different.

The absolute dimensions of the diploid and tetraploid shoot apices were used to compute certain other size relationships. From the basal diameter may be computed the basal circumference of the shoot apex (table 1, item 3), which is also a measure of the lateral extent of the leaf primordium at the time of its inception, since the young leaf primordium in maize originates as a circumferential structure at the base of the shoot apex. From a comparison of the computed basal circumferences of the diploid and tetraploid, it was apparent that the basal circumference of the tetraploid was 1.19 times that of the diploid. This is an important relationship, since it determines the relative widths of the leaf primordia at the time of their origin. As will be shown later, there is a close correlation between the width or circumferential extent of the leaf primordium in the diploid and the tetraploid and the ratio (1.16) between the width of the tetraploid leaf and that of the diploid, for any given length during their subsequent development.

The absolute dimensions of the diploid and tetraploid shoot apices were used also in computing their relative volumes. The shape of the shoot apex is that of a paraboloid of revolution. By utilizing the formula for determining the volume of such a structure (*2*), as shown in table 1, item 5, it was found that the volume of the tetraploid was 1.81 times that of the diploid, a value which is equivalent on a linear basis to 1.22 ($\sqrt[3]{1.81}$) times. The shoot apex is in itself an important morphological entity during an extended period in the ontogeny of the plant. Since various organs of the mature plant are derived from the shoot apex, the fact that polyploidy directly influences its volume relations is of primary importance in any consideration of the developmental relations of these organs.

Since the tetraploid shoot apex was larger than that of the diploid (fig. 1 and table 1), the question arose as to the relation of cell number and cell size to organ size. The cells were counted in the basal diameter of median longitudinal sections of diploid and tetraploid shoot apices. In the diploid the average number of cells was 10.1, and in the tetraploid 9.9 (table 1, item 8). There was no statistically significant difference between these two values. By dividing the basal diameter of the shoot apices by the number of cells in the basal diameter it was determined that the average diameter of the diploid cells was 12.9μ and the average diameter of the tetraploid cells was 15.6μ (table 1, item 9), the average diameter of the tetraploid cells being 1.21 times that of the diploid.

The average area of individual cells in the diploid and tetraploid shoot apices was computed by dividing the average median longitudinal area of the shoot apex by the average number of cells in the median longitudinal plane of section. The resultant values, computed on the assumption that the median plane of section had the form of a paraboloid, are given in table 1. The average sectional area of the tetraploid cells was 169μ^2 and that of the diploid cells was 104μ^2. The average number of cells in the median plane of section was 56.8 for the tetraploid and 60.8 for the diploid, there being no statistically significant difference between these two numbers. However, the average area of the tetraploid shoot apex in median longitudinal section was approximately 50 percent greater than that of the diploid, the actual values being 9,600μ^2 and 6,300μ^2, respectively. The average area of the individual tetraploid cells in the median longitudinal plane of section was 1.63 times, or on a linear basis, 1.28 ($\sqrt{1.63}$) times that of the individual diploid cells (table 1, item 7). This ratio is in reasonably good agreement with that obtained for the comparison of cell diameters.

The average cell volume in the diploid and in the tetraploid shoot apex, obtained by multiplying cell area by cell diameter (table 1, item 10), was found to be in the tetraploid nearly twice (1.96 times) that in the diploid, which corresponds to a linear relationship of 1.25 ($\sqrt[3]{1.96}$) times.

It was anticipated that chromosome doubling would affect nuclear size as well as cell size. Nuclear volume, computed from direct measurements of nuclear diameter (table 1, item 12), was found to be twice (actually 1.99 times) as great in the tetraploid as in the diploid. This was to be expected, since the tetraploid nuclei contained twice as many chromosomes as the diploid.

From these computations of nuclear and cell relations it was apparent that in the shoot apex chromosome doubling resulted in a doubling of nuclear and cell volume without any change in cell number or cell pattern, and the volume of the tetraploid shoot apex was also essentially twice as great as that of the diploid. But this simple relationship did not prevail in the mature leaf blade, either in the form or pattern of the leaf as a whole or in the cells and tissues of which it was composed, as will be shown later.

DEVELOPMENT OF THE TENTH LEAF BLADE

In the initial stages of development the tenth leaf of the tetraploid was greater in width than that of the diploid. This was clearly due to the increased size of the tetraploid shoot apex. In maize the primordium of the leaf develops as a ridge that almost completely surrounds the base of the shoot apex, and since the circumference of the tetraploid shoot apex was greater than that of the diploid there was a corresponding increase in the width of the embryonic tetraploid leaf. In tracing the subsequent development of the leaf it was at once apparent that this initial advantage in width of the tetraploid was not maintained in terms of amount of growth in width per unit of time. The absolute and relative dimensions of the leaf blade at maturity were also indicative of altered developmental relationships.

RATE OF INCREASE IN WIDTH AND LENGTH

Since the relation of one dimension to another in development (see fig. 5) is a reflection of the rate at which each of the dimensions changes (figs. 2 and 3), it seemed desirable first to consider separately the rate of increase in length and in width of the diploid and tetraploid maize leaves.

The rates of increase in the length and width of the tenth leaf are represented in figure 2, *A* and *B*, respectively. In each figure the

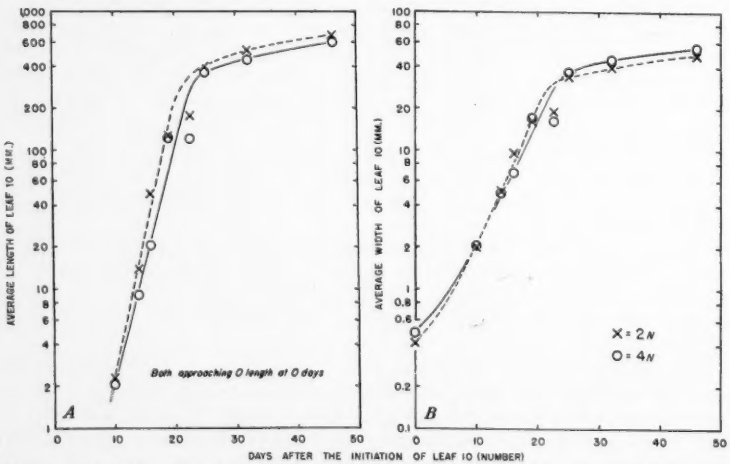

FIGURE 2.—Growth rate of leaf 10 in (*A*) length and (*B*) width in diploid and tetraploid maize. The dimension of the leaf is plotted logarithmically on the ordinate. Time is plotted arithmetically on the abscissa.

linear dimension (on the ordinate) is plotted logarithmically. The size of the sample taken on any one day averaged 20 leaves (varying from 16 to 24), the plants being chosen to include the greatest range in size for a particular day, except for the twenty-second and twenty-third days after the origin of the leaf initial when only smaller leaved plants were taken. The latter exception explains the deviation from the trend exhibited by the values for that collection (fig. 2, *A* and *B*).

The rates of increase in length (fig. 2, *A*) of the diploid and tetraploid leaf blades differed slightly, and the time at which they approached maturity was also somewhat different. In measuring leaf elongation during ontogeny both diploid and tetraploid tenth leaves may be considered to start at zero length. Subsequently the tetraploid elongated at a slightly lower rate than the diploid, so that its absolute length as development proceeded became a smaller and smaller proportion of the length of the diploid at the same harvests (table 2). But the diploid, after having grown more rapidly in length, also began to mature sooner (see "break" in curve for 2*n*, fig. 2, *A*), so that the tetraploid leaf, through a later inception of the maturation process, nearly reached the length of the diploid at maturity (fig. 2, *A*, and table 2). In other strains, studied only at maturity (table 3), it was found that the tetraploid may surpass the diploid in its mature leaf length. In the growth of the leaf blade in length there are, then, at least two important factors, which may well be independent variables (perhaps subject to separate genic control), one being the absolute growth rate and the other the threshold of maturation.

TABLE 2.—*Ratios of widths and of lengths of the tenth leaves of tetraploid and diploid maize plants at different stages of development* [1]

Dimension	Ratio (4*n*/2*n*) at indicated number of days from initiation of leaf								
	0	10	14	16	19	22–23	25	32	46
Length	.....	0.89	0.65	0.43	0.96	0.68	0.93	0.85	0.90
Width	1.19	1.02	.97	.72	1.06	.86	1.08	1.11	1.14

[1] Based upon a total of 316 leaves of each type.

TABLE 3.—*Length and width of mature tenth leaves of diploid and tetraploid maize*

Strain	Stock		Where grown	Leaf length			Leaf width			
	Diploid	Tetraploid		Diploid	Tetraploid	Ratio (4*n*/2*n*)	Diploid	Tetraploid	Ratio (4*n*/2*n*)	Leaves measured
				Cm.	*Cm.*		*Cm.*	*Cm.*		*Number*
A	3760A	3759A	Greenhouse.	67.2±[1]2.5	60.2±2.3	0.90	4.8±0.2	5.5±0.2	1.15	26
A′	3760A	3759A	Field	73.8± 3.9	70.9±3.8	.96	4.7± .2	5.3± .2	1.13	22
B	41410	41409	do	82.4± 1.6	83.7±2.5	1.02	9.5± .3	11.5± .4	1.21	20
C	41437	41436	do	73.8± 1.3	81.7±2.2	1.11	9.5± .2	10.9± .3	1.15	20
D	41459	41457	do	82.6± 1.0	82.2±1.6	1.00	9.1± .3	11.0± .2	1.21	20
E	41449	41446	do	65.4± .9	66.9±1.0	1.02	9.3± .2	10.0± .4	1.08	20
Mean	.....	.....	.....	.....	.....	1.00±.03	.....	.....	1.16±.02	.....

[1] Standard error.

The rates of increase in the width of the leaf (fig. 2, *B*) also differed in the diploid and the tetraploid. Basically the relationships between the curves are like those for length except that the starting points for the width comparisons differ, whereas the starting points for the length comparisons are the same. The tetraploid leaf blade increased in

width more slowly than the diploid and entered the maturation phase somewhat later. The tetraploid leaf primordium was wider than the diploid, but this initial advantage was not maintained by the developing leaf blade. It remained wider for a relatively brief period. Then, because of its slower growth rate, its width approached that of the diploid and then fell below it, but ultimately surpassed it again because of the later onset of maturation (fig. 2, *B*, and table 2). At maturity, the width of the tetraploid leaf was 1.14 times that of the diploid, nearly equivalent to its initial width advantage (1.19 times); but in the intervening period it had fallen below and again surpassed the diploid. Thus, the initial size of the organ, growth rate, and maturation threshold are important factors influencing form determination.

The rates of increase in width and in length show a basic similarity in the relative behavior of the diploid and tetraploid leaf blades. In comparison with the diploid, the rate of increase of the tetraploid leaf blade is slower for both width and length, but this is partly compensated by the delay in the time of maturation.

INCREASE IN WIDTH AS RELATED TO INCREASE IN LENGTH

The marked similarity between the rates of growth in length and in width of the tetraploid (and also of the diploid) is clearly apparent not only when the single dimension is considered in relation to the time factor (fig. 2, *A* and *B*), but also when the dimensions are considered in relation to each other (fig. 3, *A* and *B*). The data are plotted arithmetically in figure 3, *A*, and logarithmically in figure 3, *B*, the latter having the advantage that the parallel nature of the curves makes visually evident the fact that, for any given length, the tetraploid leaf is wider than the diploid by a uniform percentage. It is thus obvious that while the tetraploid leaf blade grows more slowly than the diploid, width increases in proportion to length in the same way that it does in the diploid; for any given increase in length there was a proportional increase in width in both the diploid and the tetraploid. However, the tetraploid leaf started as a structure of greater lateral extent, while length increase started at essentially zero in each case. The tetraploid leaf blade had an initial advantage in width (1.19 times), which was maintained at about the same level (1.16 times) throughout development. It should be kept in mind that this is a comparison of one dimension with another, in contrast to the rate (dimension against time) comparisons described under the preceding heading. The growth in width relative to length was, then, similar in the diploid and the tetraploid, but for any given width of the diploid at a given length the tetraploid leaf at that same length was on the average 1.16 times as wide. This value was based on measurements of 316 leaves representing various developmental stages from a length of 1.5 mm. to maturity. The measurements were seriated on the basis of length, and the corresponding lengths and widths were averaged in groups of 10, beginning with the smallest. These averages were plotted arithmetically (fig. 3, *A*). By means of this arithmetic graph (in which the amount of distortion in the central and upper portions was less than in the logarithmic graph), the average widths of the diploid and tetraploid leaves were determined, by graphic interpolation at length intervals of 100 mm., in leaves ranging from

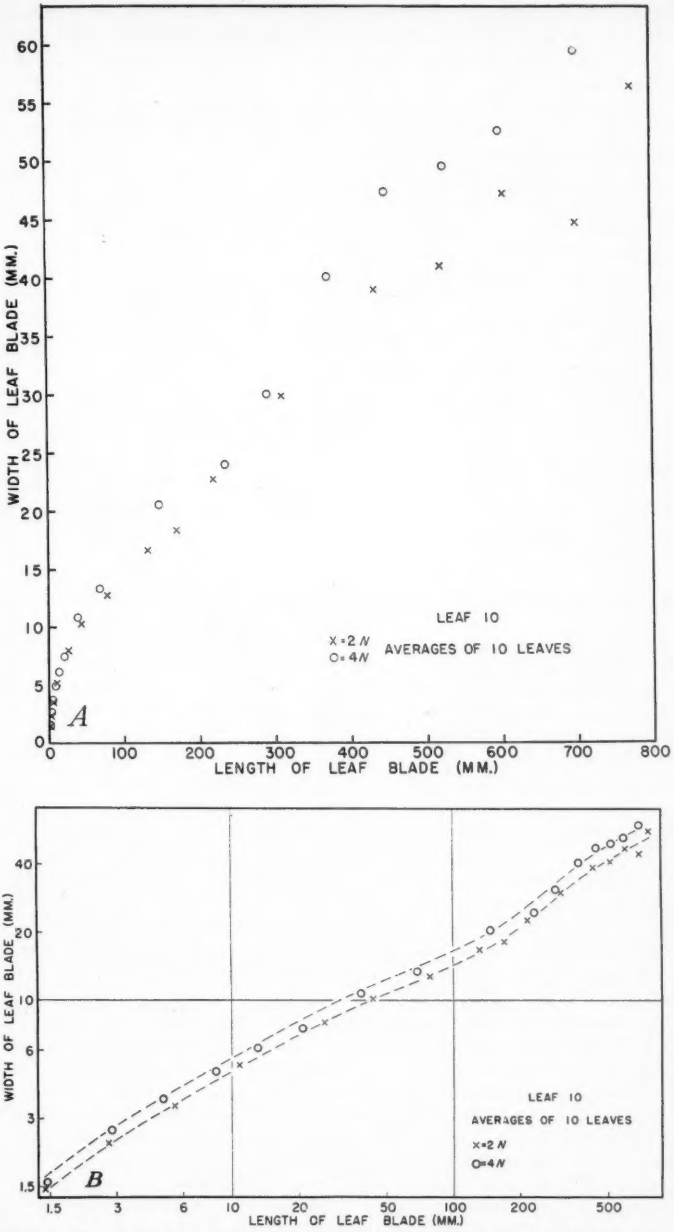

FIGURE 3.—Relation of leaf width to length during development in diploid and tetraploid maize, plotted (*A*) arithmetically and (*B*) logarithmically.

100 mm. to 700 mm. in length. The ratio of the width of the tetraploid leaf to that of the diploid for a given length was computed for each length class and was found to be on the average 1.16 ± 0.03. There was relatively little departure from the average at any stage in development. It is undoubtedly significant that this value is essentially the same as the ratio (1.19) between the width of the tetraploid leaf initial and that of the diploid.

The results of the study of the developmental patterns of the diploid and tetraploid leaf blades may be summarized as follows. (1) For any unit of time, the diploid leaf blade increased more than the tetraploid in length and width and also started to mature sooner. At maturity the tetraploid approached the diploid very closely in length, although it was definitely shorter than the diploid during intermediate stages of development; and in width the tetraploid surpassed the diploid at maturity, having started at a greater width but fallen behind during its earlier development. (2) The slower rate of increase in width and length of the tetraploid is of such a nature that the relative increase in width for a given increase in length is very similar to that in the diploid. Thus, for any unit of length increase, there is a proportional increase in width in both the diploid and tetraploid, but the width of the tetraploid is 1.16 times that of the diploid, irrespective of stage of development, this being closely correlated with the initial advantage (1.19 times) in width of the tetraploid leaf primordium.

GROSS MORPHOLOGY OF THE MATURE TENTH LEAF BLADE

The external form, as well as the internal cellular organization, of the mature tenth leaf was compared in five different strains (A, B, C, D, and E, table 3) and in a sectorial chimera in which comparable diploid and tetraploid material was available. Strain A was the same strain as that used in the studies of the shoot apex and leaf development previously described, and both greenhouse-grown (A) and field-grown (A′) diploid and comparable tetraploid plants were examined. The tetraploid strains B, C, and D originated from a chromosome-doubled hybrid involving diploid inbred lines of Illinois A, Webbers Dent, and Luces Favorite, the three strains being segregated as separate lines after three generations of inbreeding and thereafter self-pollinated for an additional four generations. The tetraploid strain E originated from a chromosome-doubled hybrid of two morphologically similar lines of Spanish Flint, which was subsequently inbred for five generations. Diploid stocks of each of the four tetraploid strains B, C, D, and E were obtained from the selfed progeny of parthenogenetic diploids, which arose as spontaneous mutants in the tetraploid strains during the generation immediately preceding that in which the comparisons were made. (See Randolph and Fischer (*9*) for a discussion of parthenogenesis in tetraploid maize.) In addition to the studies of the tenth leaf in these five strains, the third leaf of a plant that was half diploid and half tetraploid and had appeared in a heat-treated genetic culture was also studied (fig. 4). One-half of each leaf of this plant was tetraploid and the other half diploid, the line of demarcation between the two being in the region of the midrib. This chimera furnished ideal material for a critical comparison of diploid and tetraploid leaf tissue, since the possibility

of the existence of genetic or environmental differences in such material could be disregarded. The tenth leaf in the mature plants of strain A ordinarily subtended the functional ear shoot, and this criterion

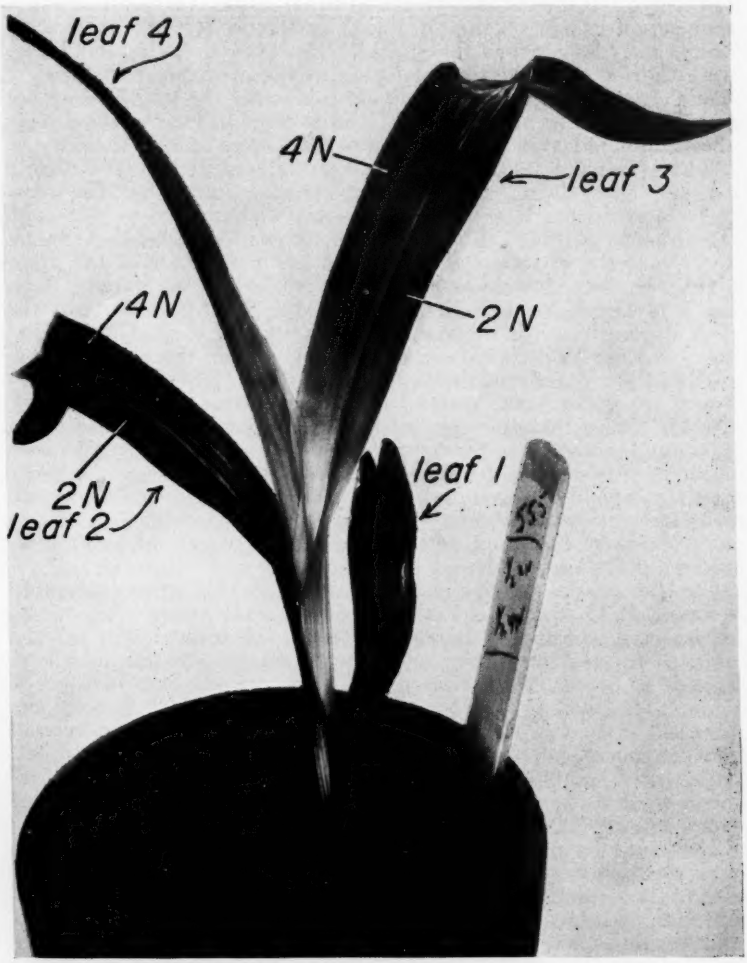

FIGURE 4.—A sectorial chimera in maize. The portion of each leaf labeled "4*n*" is tetraploid from the edge to the midrib and for the full length of the leaf, while the rest of each leaf (labeled "2*n*") is diploid.

was utilized in selecting leaves for measurement from the mature plants of strains B, C, D, and E.

The length and width measurements of the tenth leaf in strains A, A′, B, C, D, and E are summarized in table 3. The leaves of both the diploid and tetraploid plants of strain A were notably narrower for their length than those of the other strains. This

might have been due partly to the fact that this strain was not planted until July 1, whereas the others were planted about May 15. The lengths of the tetraploid and diploid leaves were very similar in four of the five sets of comparisons made on full-grown plants; in the fifth comparison (strain C) the tetraploid leaf blades were on an average significantly longer than the diploid. However, the tetraploid leaves were shorter than the diploid leaves in the greenhouse material of strain A; in the field-grown plants of this strain the lengths were not significantly different. The combined average lengths of the diploid and tetraploid leaves for all six comparisons were exactly the same.

The tetraploid leaves were significantly wider than the diploid leaves in all of the strains, the average increase in width of the tetraploid leaves being 1.16 times that of the diploid leaves. Although the absolute widths of the leaves in the later planted field and greenhouse material of strain A were much less than those of the other four strains, which were planted at the earlier normal planting date, the tetraploid leaves exceeded the diploid in width to essentially the same degree in all of the strains. It is especially noteworthy that the tetraploid leaves were consistently wider than the diploid leaves in all of the comparisons that were made. Furthermore, in these five strains the mean width ratio of the tetraploid mature leaves to the diploid (1.16) coincided very closely with the average value obtained for the entire ontogenetic development of the leaf in strain A. Because of the greater size of the shoot apex, the tetraploid leaf was wider than the diploid in the initial stages of development, and there was a similar consistent difference in the widths of the diploid and tetraploid leaves at maturity, although their lengths at maturity were essentially the same.

The thickness of the diploid and tetraploid leaf blades was measured in strains B, C, D, and E from either hand sections or frozen microtome sections of freshly harvested leaves, the measurements being taken in the region midway between the margin and the midrib of the leaf at a point approximately 15 cm. from the base of the leaf blade. The thickness of the diploid and tetraploid halves of the third leaf of the sectorial chimera previously mentioned was measured from permanent mounts, the leaf tissue having been fixed in alcohol, sectioned in paraffin, and stained with haematoxylin. All measurements were made with an ocular micrometer at a magnification of approximately 330, which permitted a very high degree of accuracy.

There was an unexpected uniformity in the absolute thickness of both the diploid and the tetraploid tenth leaf blades (table 4). In each of the four strains the average thickness of the diploid leaves was 0.26 mm. and the average thickness of the tetraploid leaves 0.35 mm. Thus the tetraploid tenth leaf was 1.35 times as thick as the comparable diploid leaf. This striking uniformity in leaf thickness in the different diploid and tetraploid strains is remarkable, in view of the differences in the average lengths and widths of the leaves in these same strains, and suggests that leaf thickness in maize is less subject to genetic variation than are the two other dimensions of the leaf.

The diploid and tetraploid halves of leaf 3 of the sectorial chimera (table 4) were somewhat thinner than the comparable regions of the tenth leaves of strains B, C, D, and E, and the tetraploid half was only 1.2 times as thick as the diploid half.

TABLE 4.—*Comparison of leaf thickness in mature diploid and tetraploid leaf blades*

Strain	Stock		Type of section	Leaf No.	Thickness of leaves at maturity						Ratio (4n/2n)
					Diploid			Tetraploid			
	Diploid	Tetraploid			Leaves measured	Measurements of thickness	Mean thickness and standard error	Leaves measured	Measurements of thickness	Mean thickness and standard error	
					Number	*Number*	*Millimeter*	*Number*	*Number*	*Millimeter*	
B	41409	41410	Hand	10	5	100	0.26±[1]0.10	5	100	0.35±[1]0.01	1.35
C	41437	41436	do	10	5	100	.26± .01	5	100	.35± .01	1.35
D	41459	41457	Frozen	10	10	545	.26± .01	10	335	.35± .01	1.35
E	41449	41446	Hand	10	5	100	.26± .01	5	100	.35± .01	1.35
Average							.26			.35	1.35
Chimera	A70	A70	Paraffin	3	1	49	.13± .01	1	65	.15±.003	1.15

[1] Standard error of individual means.

The length, width, and thickness of the tenth leaf blade having been determined, it was possible to compute the approximate volume of the diploid and tetraploid leaves in the various strains. The surface of the tenth leaf blade at maturity closely approximated the form of an isosceles triangle, and for the purpose of volume comparisons it was assumed that the leaf has a uniform thickness throughout, although in this study sufficient measurements were not taken in different regions of the leaf to verify this assumption.

The difference in the volumetric relations of the mature diploid and tetraploid leaves was not so great as was the difference in the volume of the shoot apices. The volume of the tetraploid shoot apex was, on an average, 1.80 times that of the diploid shoot apex, whereas the volume of the mature tenth leaf in the tetraploid plants was 1.54 times that of the comparable leaf in the diploid plants. These differences in volume relations were presumably related to the differences in the response of the various dimensions of the mature leaf blade to chromosome doubling. On a linear basis the average width of the tetraploid leaf was 1.16 times that of the diploid and its thickness was 1.33 times that of the diploid, whereas their lengths were the same. Expressed on a linear basis the volume of the tetraploid shoot apex was 1.22 times the volume of the diploid shoot apex. Chromosome doubling resulted in an essentially symmetrical size increase of the shoot apex and an asymmetrical increase in the various dimensions of the mature leaf blade. The manner in which the quantitative size relations in the diploid and tetraploid leaves were related to the cellular organization and internal structure of the leaf remains to be discussed.

HISTOLOGY OF THE MATURE TENTH LEAF

The tenth leaf blade of maize has a definite cell pattern when viewed in cross section (fig. 5). The upper and lower epidermal tissues are each one cell layer in thickness. Their component cells are elongated parallel to the long axis of the leaf blade and tend to be slightly wider (in dimension parallel to the leaf surface and perpendicular to the

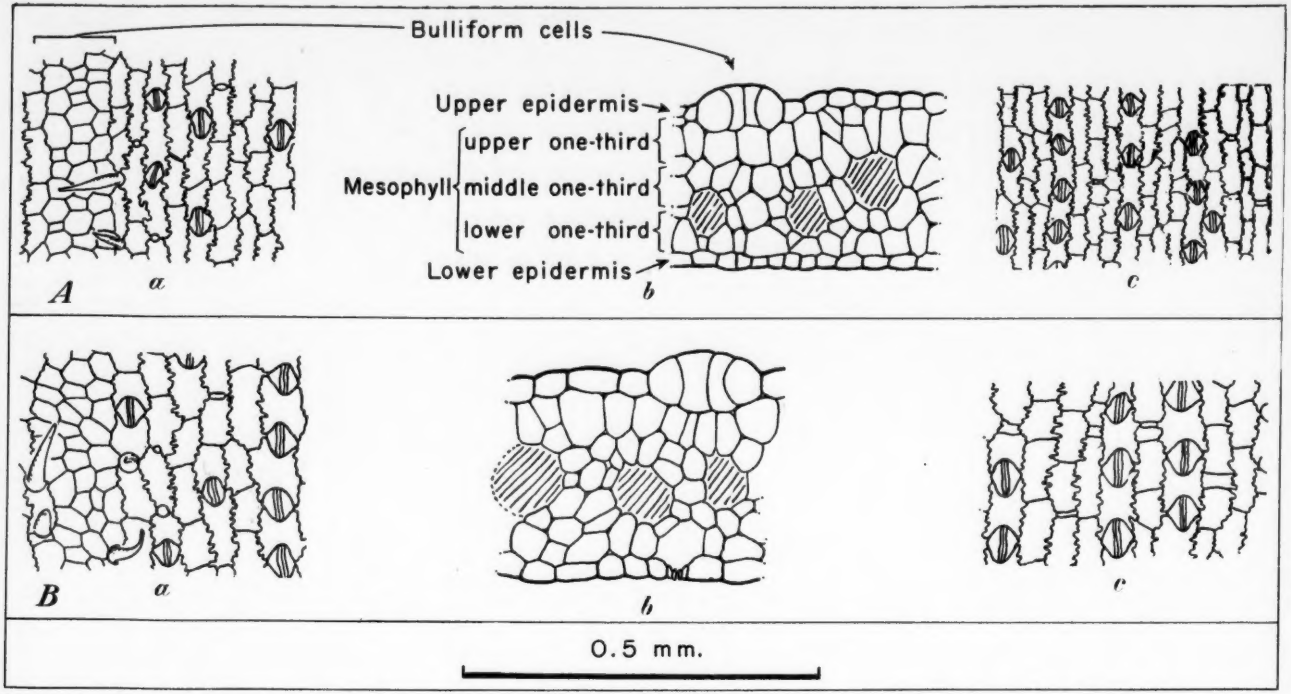

FIGURE 5.—Relative dimensions of cells in the epidermis and mesophyll of diploid (*A*) and tetraploid (*B*) maize. *a*, Upper epidermis, face view; *b*, cross section; *c*, lower epidermis, face view. Camera lucida drawings. Cross hatching indicates vascular bundles.

midrib) than they are thick (in dimension perpendicular to the leaf surface). The upper epidermis is provided also with long rows, several cells in width, of essentially isodiametric and somewhat distended bulliform cells. Along the edges of these areas of bulliform cells are occasional unicellular, conical hairs. Stomata are interspersed in certain rows of the epidermal cells. The lower epidermis resembles the upper except that bulliform cells are absent, the stomata are more abundant, and the cells tend to be smaller than in the upper epidermis. The mesophyll is composed of relatively unspecialized parenchyma cells, which differ markedly in size and have only occasional, very small intercellular spaces. Vascular bundles of various sizes are located within the mesophyll.

For the determinations of cell number and cell size the mesophyll was subdivided for convenience into the upper, middle, and lower thirds. The cells of the lower and middle thirds were of about the same size, but the presence of numerous vascular bundles tended to modify the form of the cells in the middle third. Those of the lower third were more nearly symmetrical. The cells of the upper third of the mesophyll were larger than those of the middle and lower third. Strain D was used for the most complete series of measurements of the cells of the leaf, since the relative length, width, and thickness of the diploid and tetraploid leaves of this strain most nearly approximated the averages for all of the strains that were studied (tables 3 and 4). Strain E was used in a few cases for an additional comparison. The cell measurements were made so as to avoid vascular bundles and bulliform cells. In measuring the length and width of the epidermal cells, groups of 8 to 12 cells from cell rows containing no stomata were measured as a unit to reduce experimental error. Elsewhere, individual cells were measured. The epidermal cells were measured with an ocular micrometer from hand sections. The mesophyll cells were measured from projected drawings of frozen microtome sections.

The mesophyll averaged 5.4 cells in thickness in the diploid and 6.2 cells in the tetraploid, the determinations being based on counts from 45 sections taken in the region of the leaf 15 cm. from the base and midway between the midrib and the margin. It is probable that this difference in cell number was not significant. The mesophyll in the third leaf of the sectorial chimera previously mentioned averaged 3.8 cells in thickness for the tetraploid sector and 3.4 for the diploid sector, indicating that there was no significant difference between the diploid and the tetraploid with respect to the number of cells in the cross-section area of the mesophyll tissue. These data from the chimeral leaf also suggest that the earlier formed leaves of the maize plant have fewer mesophyll cells in cross section than do the later leaves. This fact emphasizes the importance of comparing morphologically homologous organs in quantitative analyses of form differences.

The length and width of the cells of the lower epidermis were measured at distances of 15 and 30 cm. from the base of the leaf blade in strains D and E (table 5) to determine the relation of location along the longitudinal axis of the leaf to cell size. A series of measurements were made also at 50 cm. from the base in strain D to check further on the position effect (table 5). It was immediately evident that the length and width of the lower epidermal cells fluctuated markedly with their position in the leaf. Thus, in strain D the absolute length of the cell, in both the diploid and the tetraploid, increased with the distance

between the cell and the base of the leaf. This was true also in the diploid leaf of strain E, but the reverse was the case in the tetraploid leaf of strain E. The width of the lower epidermal cells did not change appreciably with position in strain E, but it did vary somewhat in strain D, although not in any very significant manner. The length of the epidermal cells appears to change with position in the leaf to a greater absolute extent than does their width. Chromosomal constitution likewise has a major effect on the dimensions of the epidermal cells.

TABLE 5.—*Dimensions of cells in epidermis and mesophyll of the tenth leaf in diploid and tetraploid maize*

Tissue, cell dimension, strain, and distance above ligule (cm.)	Diploid (2*n*)			Tetraploid (4*n*)			Ratio of cell dimension (4*n*/2*n*)
	Average cell dimension and standard error	Cells measured	Leaves sampled	Average cell dimension and standard error	Cells measured	Leaves sampled	
Lower epidermis:							
Length [1]—	μ	*Number*	*Number*	μ	*Number*	*Number*	
D (15)	73±1	2,108	10	82±3	1,837	10	1.12
E (15)	83±3	873	5	101±5	730	5	1.22
D (30)	87±4	556	2	98±8	506	2	1.13
E (30)	98±2	492	2	88±3	553	2	.90
D (50)	104±4	154	1	95±3	168	1	.91
Width [2]—							
D (15)	44±2	1,900	10	44±2	1,900	2	1.00
E (15)	34±1	900	5	47±1	900	5	1.38
D (30)	49±1	600	2	46±1	600	2	.94
E (30)	36±1	600	2	47±1	600	2	1.31
D (50)	30±1	200	1	42±1	200	1	1.40
Thickness [3]—D (15)	31±1	250	5	37±1	250	5	1.19
Upper epidermis:							
Length [1]—							
D (15)	75±2	1,073	5	73±3	1,049	5	.97
D (50)	90±3	178	1	117±3	137	1	1.30
Width [2]—							
D (15)	36±2	1,000	5	49±2	1,000	5	1.36
D (50)	32±1	200	1	42±1	200	1	1.31
Thickness [3]—D (15)	39±2	50	5	48±2	50	5	1.23
Mesophyll:							
Upper third:							
D (15)—							
Width [2]	48±5	100	5	59±5	100	5	1.23
Thickness [3]	51±3	100	5	69±2	100	5	1.35
Middle third:							
D (15)—							
Width [2]	41±3	100	5	43±2	100	5	1.05
Thickness [3]	44±2	100	5	47±1	100	5	1.07
Lower third:							
D (15)—							
Width [2]	43±3	100	5	41±2	100	5	.95
Thickness [3]	45±3	100	5	48±3	100	5	1.07

[1] Diameter parallel to surface and midrib of leaf.
[2] Diameter parallel to surface and perpendicular to midrib of leaf.
[3] Diameter perpendicular to surface of leaf.

In strain D there was a definite tendency for the length of the cells in the upper epidermis to increase and for their width to decrease with their distance from the ligule, or leaf base (table 5).

The relative cell dimensions (4*n*/2*n*) varied with position in strains D and E. With respect to the length of lower epidermal cells there was a tendency for the cells of the tetraploid to be relatively longer than those of the diploid near the base of the leaf in both D and E, although the ratios were by no means the same in the two strains. However, with respect to cell width there was not even this much agreement between strains D and E. Both chromosomal constitu-

tion and position in the leaf had a varying relation to the ratio between cell length and cell width in the lower epidermis of the diploid and tetraploid leaves. Cell thickness in the lower epidermis at a distance of 15 cm. from the ligule was somewhat less than cell width in the tetraploid leaves and 1.19 times cell width in the diploid leaves.

The relative dimensions of the cells in the upper epidermis also varied, depending on position in the leaf, the length of the cells being nearly the same in the diploid and tetraploid leaves at 15 cm. from the base of the leaf but 1.3 times as long in the tetraploid as in the diploid at a distance of 50 cm. from the base. The width of the cells in the tetraploid was somewhat more than 1.3 times that in the diploid, both at 15 cm. and 50 cm. from the base. The thickness of the upper epidermal cells at 15 cm. from the base was 39μ in the diploid and 48μ in the tetraploid, the $4n/2n$ ratio being 1.21.

In the mesophyll the cells of the lower and middle thirds of the leaf were very nearly isodiametric and tended to be the same size or but slightly larger (0.95 to 1.07 times) in the tetraploid than in the diploid. The cells of the upper third of the mesophyll were, however, not only markedly larger than the rest of the cells of the mesophyll in the same leaf but also relatively much larger in the tetraploid leaves than in the diploid leaves, the ratio being 1.35.

The survey of cell size in the various parts of the diploid and tetraploid leaves of strains D and E showed a marked tendency toward independent dimensional behavior of each of the parenchymatous constituents of the leaf, and there was a pronounced variation in cell dimensions both in comparable regions of different strains and in different regions of the same strain. Although there was a tendency for the cells of a given tissue in the tetraploid leaf to be larger than the comparable cells in the diploid leaf, in another strain or in another location in the leaves of the same strain the cells of the tetraploid leaf in some cases were of the same size or even slightly smaller than the comparable cells of the diploid leaf. Furthermore, the relative size of all the cells contributing to a given dimension of a leaf was not changed in the tetraploid to precisely the same degree as the gross dimensions of the leaf were changed with respect to the comparable dimensions of the diploid leaf. Thus the length of the leaf, although it remained essentially the same in the tetraploid and diploid leaves of strain D, nevertheless represented the summation of fewer cells near the base and middle of the lower epidermis but smaller and more cells toward the outer region of the leaf of the tetraploid as compared with the diploid. Width of leaf in the tetraploid plants of strain D was 1.21 times that of diploid plants in the same strain (table 3), yet the lower epidermis had cells of the same width in the tetraploid as in the diploid at distances of 15 cm. and 30 cm. from the base of the leaf (table 5), so there must be more cells in that region of the tetraploid; whereas at 50 cm. from the base, the lower epidermis of the tetraploid leaf had fewer cells in width than did the diploid. The upper epidermis, on the other hand, had larger but fewer cells in width than would be expected from the external measurements of the leaf. The thickness of the leaf in tetraploid plants of strain D was 1.33 times that of the diploid, yet the lower epidermal cells were only 1.19 times as thick, the lower and middle mesophyll cells only 1.07 times as thick, the upper third of mesophyll

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cells 1.35 times as thick, and the upper epidermal cells 1.23 times as thick. In spite of the independence of the various cell layers, insofar as relative dimensions and rates of division were concerned, there was no visible evidence of tissue tension. The changed pattern of the leaf in the tetraploid was apparently as well integrated during development and at maturity as in the original diploid form, which suggests that the organ as a whole in some obscure manner exerts a regulatory influence over its constituent parts during ontogeny.

ESTIMATES OF TOTAL NUMBER OF CELLS IN THE MATURE LEAF BLADE

Having determined the over-all dimensions of the mature tenth leaf blade and the dimensions of individual cells in representative regions of the leaf, exclusive of the vascular tissue (table 5), in various diploid and tetraploid stocks of maize (tables 3 and 4), it was possible to estimate with a fair degree of accuracy the total number of cells throughout the various tissues of the leaf. The summation of these values would then yield an estimate of the total number of cells, exclusive of the vascular tissue, in the entire diploid and tetraploid leaf blades. So far as the writers are aware, suitable data have not previously been available from which reliable estimates of the total number of cells in the leaves of the maize plant could be made. The diploid and tetraploid stocks of strain D were utilized in making these computations, as the cell and organ dimensions in this strain closely approximated the mean for all of the strains studied. Cell dimensions at 15 cm. from the base of the leaf blade were utilized in making the computations of cell number, as a complete set of values for other regions of the leaf was not available.

Table 6 records the number of cells in the various tissues throughout the length of the diploid and tetraploid leaf blades, calculated from the dimensions obtained for the leaf blade and its constituent cells.

In making these computations it was necessary to assume that the mesophyll cells had the same length and width and that each of the three regions of the mesophyll was two cell layers thick. The cytological observations of the mesophyll tissue indicated that these were reasonable assumptions.

Table 6 also lists the number of cells in the maximum width of the various tissues of the mature leaf, calculated from the dimensions obtained for the leaf blade and its constituent cells.

The relative values for the different tissues of the diploid and tetraploid leaves were not the same. For example, there were fewer cells in the lateral extent of the upper epidermis of the tetraploid leaf than there were in the upper epidermis of the diploid leaf; but the reverse was true of the lower epidermis. This was due to the fact, previously emphasized, that different tissues often respond differently with respect to the changes in cell dimensions that result from chromosome doubling.

Leaf thickness, which was consistently 1.35 times as great in the tetraploid as in the diploid (table 4), is a dimension in which, by direct count, there was essentially the same number of cells in both stocks (7.4 ± 0.3 in the $2n$ and 8.2 ± 0.3 in the $4n$, based on counts of 45 different diameters in each).

TABLE 6.—*Number of cells in mature tenth leaf blade of diploid and tetraploid maize*

Leaf tissue	Cell rows	Cells in—	
		Diploid	Tetraploid
	Number	*Number*	*Number*
CELLS IN LENGTH OF LEAF			
Upper epidermis	1	11,000	11,300
Mesophyll:			
Upper third	2	34,400	27,900
Middle third	2	40,300	38,200
Lower third	2	38,400	40,100
Lower epidermis	1	11,300	10,000
Total	8	135,400	127,500
CELLS IN WIDTH OF LEAF			
Upper epidermis	1	2,500	2,200
Mesophyll:			
Upper third	2	3,800	3,700
Middle third	2	4,400	5,100
Lower third	2	4,200	5,400
Lower epidermis	1	2,100	2,500
Total	8	17,000	18,900
TOTAL CELLS IN LEAF TISSUES			
Upper epidermis	1	14,000,000	12,000,000
Mesophyll:			
Upper third	2	33,000,000	26,000,000
Middle third	2	44,000,000	49,000,000
Lower third	2	40,000,000	54,000,000
Lower epidermis	1	12,000,000	13,000,000
Total	8	143,000,000	154,000,000

The entire number of cells in the mature leaf blade may be computed from the foregoing data by assuming that the leaf blade is a wedge-shaped solid. The total number of cells in any tissue would then be half of the number in a rectangular solid with a corresponding number of cells in each linear dimension. On this basis, cell number in each of the tissues and the total in the whole leaf may be determined. The number of cells (to the nearest million), computed in this fashion, is shown in table 6.

The above results may be checked by taking half the product of the average number of cells in length, width, and thickness. This gives (to the nearest million) 144 million cells for the diploid and 150 million for the tetraploid, the difference between these results and those reached by the summation method being due to the way the figures have been rounded off in the process of calculation.

It is apparent from these figures that the diploid and tetraploid leaves contained approximately the same number of cells, the total number of cells in the diploid being 143 million and in the tetraploid 154 million. Considering the methods used in arriving at these estimates, the slightly larger number of cells in the tetraploid is probably not significant. These data on cell number indicate that there was a marked tendency for the diploid and tetraploid leaf blades to undergo approximately the same number of cell divisions during ontogeny, but probably at a slower rate in the tetraploid.

As the number of cells in the diploid and tetraploid leaves is essentially the same, the average volumes of the cells would have the same relationship as would the volumes of the respective mature leaves. Applying the fundamental formula used above, namely, $\frac{\text{length} \times \text{width} \times \text{thickness}}{2}$, the mean volume of the diploid leaf blade was found to be 9,800 cubic millimeters and that of the tetraploid 15,800 cubic millimeters. Thus the volume of the tetraploid leaf blade was 1.6 times that of the diploid and therefore cell volume in the tetraploid blade averaged 1.6 times that of the diploid. In this connection it should be recalled that there was a considerable diversity of cell-size relationships in the diploid and tetraploid leaf tissues in various regions of the blade. However, the pattern of the tetraploid leaf was relatively constant and differed significantly from that of the diploid in that it was consistently wider and thicker but of the same length. Apparently, cell-number and cell-size relations were adjusted somewhat differently in different regions of the leaf to conform to the modified leaf pattern of the tetraploid plant. These mature-leaf comparisons also indicate that the cells of the mature tetraploid leaf were relatively not as much larger than those of the diploid as they were at the time of initiation of the leaf in the shoot apex, although absolute size in each increased greatly during ontogeny.

COMPARATIVE RELATIONS OF DIPLOID AND TETRAPLOID SHOOT APICES AND LEAVES

Various relations between the diploid and tetraploid shoot apices and leaf blades are summarized in table 7. In these comparisons the diploid was taken as the standard of comparison (i. e., $2n$=unity), and the extent to which the tetraploid differed from it ($4n/2n$) was indicated for a number of different characteristics. The ratios, presented along with the raw data in preceding tables, are here translated into more general terms. These ratios were computed from the mean values given in the tables. Since these mean values have a range of variation, as indicated in their standard errors, the ratios derived from them are to be interpreted as arbitrary expressions of a similar range of variation. In preparing table 7, ratios falling near 1.26 were grouped together in one column (column 4, "$4n$ markedly greater than $2n$"); ratios falling near 1.00 were similarly grouped in another column (column 2); intermediates in still another column (column 3); and those much greater than $2n$ were grouped in the last column. (See footnotes 2, 3, 4, and 5, table 7.)

The group of ratios centering about the linear value 1.26 (column 4, table 7) is especially noteworthy, since this value represents the expected deviation of the tetraploid from the diploid when the only modification of the tetraploid is a doubling of volume, with the result that linear dimensions are increased by $\sqrt[3]{2}$, or 1.26. This condition may be referred to as the "ideal gigas" state, since it is essentially the state that is commonly attributed to gigas plants.

The concept of an "ideal gigas state" is very useful and may well be defined in more detail at this point. The ideal gigas state is one in which (1) the tetraploid organ or other structural unit has the same number of cells and cells of the same shape as the diploid; but (2) the volume of the constituent cells is doubled in the tetraploid in

TABLE 7.—*Linear relation (or equivalent [1]) between tetraploid and diploid (4n/2n) shoot apices and leaves*

Item	4n approximately equal [2] to 2n	4n slightly greater [3] than 2n	4n markedly greater [4] than 2n	4n much greater [5] than 2n
(1)	(2)	(3)	(4)	(5)
Origin of leaf blade				
Shoot apex				
Gross dimensions (length, width, area, volume)			×	
Cell dimensions (width, area, volume)			×	
Nuclei dimensions (width, volume)			×	
Leaf initial, lateral extent			×	
Mature leaf blade				
Gross dimensions (mean for all 5 stocks)				
Length	×			
Width		×		
Thickness			×	
Volume		×		
Cell dimensions [6]				
Length				
Upper epidermis		15D		50D
Lower epidermis		50D; 30E	15D; 30D; 15E	
Width				
Upper epidermis			50D	15D
Mesophyll				
Upper third			15D	
Middle third		15D		
Lower third	15D			
Lower epidermis	15D; 30D	15E	30E	50D
Thickness				
Upper epidermis			15D	
Mesophyll				
Upper third				15D
Middle third		15D		
Lower third		15D		
Lower epidermis			15D	

[1] Ratios between areas are represented by their square roots; between volumes by their cube roots.
[2] 0.89 through 1.03, mean 0.96.
[3] 1.04 through 1.18, mean 1.11.
[4] 1.19 through 1.33, mean 1.26.
[5] Over 1.33.
[6] The number indicates the distance in millimeters from the base (ligule) of the leaf blade; the letter designates the strain.

proportion to the doubling of the volume of the nucleus, due in turn to the doubling of the number of chromosomes. The result of (1) and (2) is that (3) the external form of the tetraploid structural unit is the same as that of the diploid, but there is an increase in its size such that the total volume of the tetraploid is doubled, its surface area is greater by $\sqrt{2}$, or 1.41 times, and its linear dimensions are greater by $\sqrt[3]{2}$, or 1.26 times. This establishes a convenient, although admittedly arbitrary, definition of the morphological nature of the ideal gigas structure. The definition is based on the assumption that differences in form between tetraploids and diploids may be traced very simply to a mechanical doubling of nuclear volume and that developmental relations are not otherwise affected by doubling the number of chromosomes and genes. This definition ignores the possibility that the fundamental size relation between cell and nucleus may be the relation of volume to area. Also ignored are (1) the possible influence of absolute cell size on the realization of genically controlled form and (2) the altered functional activity of the genes when present in the doubled number.

The state in which the tetraploid closely approximates the diploid (column 2, table 7) represents a maintenance in the tetraploid of the condition typical of the diploid and is the least likely expectation

when dimensions are being compared, if mechanical increase in cell size is the only biologically significant difference between the tetraploid and diploid plants.

It is apparent from table 7 that the ideal gigas state is closely approached in the shoot apex; but in the mature leaf, departure from the ideal gigas state is characteristic of the various tissues of the leaf blade and the relation between the diploid and tetraploid cell dimensions is extremely variable. For example, in strain D, at 15 cm. from the ligule (see values indicated in boldface in table 7), cell width in the tetraploid leaf blade was approximately equal to that of the diploid in the lower epidermis and in the lower third of the mesophyll; in the middle third of the mesophyll cell width was slightly greater in the tetraploid than in the diploid; in the upper third it was markedly greater in the tetraploid; and in the upper epidermis it was much greater in the tetraploid than in the diploid. Similarly for other cell dimensions the tissues in the same and different regions of the diploid and tetraploid leaves varied widely and in a wholly unpredictable manner. In the fully differentiated tissues of the mature leaf there was no consistent relation between the dimensions of the organ and its constituent cells such as existed in the undifferentiated tissues of the shoot apex. These observations suggest that the cell exerts a more potent influence in determining organ pattern in the embryonic shoot apex than it does in the more highly specialized leaf blade.

DISCUSSION

The fact that the mature tetraploid organ seldom has the perfect gigas form as here defined has already been emphasized by several investigators. Von Wettstein (*13*) described in detail the variability in morphological constitution, not only from "Sippe" to "Sippe" on a genetic basis but also from "Rasse" to "Rasse" on a valency basis, within each of a number of moss species. He stated that the quantitative changes in chromosome mass of the original cells result in the most diverse morphological changes, often out of proportion, which can only be demonstrated on an experimental basis to be the result of increase in chromatin mass. In a study of members of the polyploid series in *Datura stramonium*, which was limited chiefly to a consideration of mature organs, Sinnott, Houghtaling, and Blakeslee (*12*) concluded that the primary differences between the members of the series were due to an increase in cell size associated with an increase in chromosome number, but they found many deviations from the perfect gigas state in specific tissues. Müntzing (*6*), in reviewing and correlating the large number of isolated cases described in the literature on polyploidy up to 1936, pointed out that autotetraploids are in general larger and have larger cells but that the differences are not quantitative, that is, the mature organs are not typically gigas in form. Polyploidy has not been studied extensively in animals, but an extremely interesting series of polyploid larvae in the newt *Triturus* was described by Fankhauser (*5*). The larvae of autotriploid newts had the same gross dimensions as the diploids, but they had larger and, except in some tissues, fewer cells. This tendency of the triploid to maintain the proportions of the diploid was attributed by Fankhauser to some phenomenon of regulation whose mechanism is at

present unknown. None of these workers made ontogenetic comparisons of the tetraploid and diploid or studied the relation of the embryonic tissues to the ultimate form of the organs derived from them.

The development of the leaf in diploid and tetraploid maize was investigated by Abbe, Randolph, and Einset (*1*), who showed that for any given length the tetraploid leaf was broader than the diploid. They showed also that this condition was related to the greater size of the tetraploid shoot apex, this greater size in turn being due to larger cells rather than to more cells. Only the tenth leaf blade was studied in relation to the shoot apex while the latter was in the tenth plastochron, and it was considered very important that the comparison be restricted to identical plastochrons because of changes in absolute and relative dimensions through successive plastochrons. This viewpoint has recently been elaborated more fully by these writers (*2*). Subsequently, Sinnott, Blakeslee, and Franklin (*11*) studied the influence of tetraploidy on form in the ontogeny of the fruit of four strains of cucurbits in which genetically comparable tetraploid and diploid races were available. They found that the tetraploid in two of the four strains had larger fruit primordia, and in all four strains the volume of the cells in the primordia was doubled, although at maturity the size relations of the tissues and fruits varied from line to line.

In a comparison of tetraploid and diploid shoot apices of *Vinca rosea*, Cross and Johnson (*3*) reported recently that the increased size of the tetraploid apex was due to an increase in cell width unaccompanied by any significant increase in cell thickness. Dr. Cross [3] very kindly provided the additional and pertinent information that the shoot apices listed by Cross and Johnson (*3*, *table 1*) were all in the fourth plastochron, except diploid apex "4," which was in the fifth plastochron, and that the original diploid strain had been inbred for 14 years. The quantitative data included (*3*, *table 1*) should therefore be recognized as representing thoroughly comparable material, both morphologically and genetically.

It is apparent from these developmental studies of the effects of polyploidy on the form and cellular organization of mature organs and the primordia from which they originate that the morphological consequences of chromosome doubling are variable and presumably depend upon the genotype involved in particular strains or species. In this recently developed field of morphogenetics it seems unwise to attempt generalizations until additional observations are available, but in quantitative studies of this sort one cannot overemphasize the importance of obtaining adequate data from strictly comparable diploid and tetraploid material that is genetically homogeneous and morphologically homologous.

The various degrees of departure from the ideal gigas state in the mature leaf blade of the writers' material raise a number of considerations: (1) The relation of cell number to organ size; (2) the relation between growth rate and the rates of increase in cell number and cell size; (3) the possible influence of absolute cell size on the rate of cell division; (4) the development of functional specialization of the maturing tissues of the leaf; and (5) evidence of genetic modi-

[3] Correspondence, January 7, 1942.

fication of any tendency to maintain the ideal gigas state throughout ontogeny.

Cell number and cell size as compared with organ size are of significance, since the interrelationship of these factors is a means of defining cell pattern as it contributes to organ form. Thus, in the shoot apex of the tetraploid as compared with that of the diploid, during the tenth plastochron, there is the same number of cells in median section and in basal diameter. Furthermore, cell volume is practically doubled in the tetraploid shoot apex. Therefore, the pattern of cell arrangement and the proportions of the cells characteristic of the diploid have been retained in the tetraploid. The tetraploid also retains the same general external form of the shoot apex as the diploid. The shoot apex of the tetraploid thus serves as an excellent and diagrammatic example of a perfect gigas organ. Because of its intimate relationship with the shoot apex, the leaf primordium may be assumed to share with the shoot apex this perfect gigas character. However, when the mature leaf blade is analyzed in a similar manner, it is apparent that the tetraploid is very different from the diploid with respect to both external form and the dimensions of the cells comprising the various tissues of the leaf.

There is no evidence that the tetraploid leaf in itself is abnormal structurally or functionally, since in relation to the diploid leaf it displays no consistent change in its cellular constitution or general form. Starting with the length of the leaf, one finds that the mature tetraploid and diploid leaves tend to be of essentially the same length in most of the stocks studied, but the tetraploid leaf is consistently thicker and is usually broader than the diploid leaf. Furthermore, the cell pattern of the diploid leaf is not consistently retained in the tetraploid for any of the regions of either the upper or lower epidermis, nor is there a general agreement between different stocks in the manner in which the diploid pattern fails to be maintained in the tetraploid. Although the diploid and tetraploid leaf blades differed significantly in external form with respect to the total number of cells, the mature tetraploid leaf blade displayed perfect gigas characteristics, in that it had the same number of cells as the diploid; but in other respects the requirements of the perfect gigas state were not met. As is evident from table 7, the constituent cells of the tetraploid leaf blade did not have the same proportions as the corresponding cells of the diploid. Furthermore, the total volume of the tetraploid mature leaf blade was not double that of the diploid but was only 1.6 times that of the diploid. Since the volume of the tetraploid mature leaf blade was 1.6 times that of the diploid and the number of cells was essentially the same in both, it is evident that the volume of the hypothetical "average" cell in the tetraploid was 1.6 times that of the diploid. Thus the second requirement of the perfect gigas state, namely, that the volume of the cells be doubled, also failed to be realized in the mature tetraploid leaf blade. The interaction of these factors is such that the external form of the tetraploid also fails to meet the requirements of the ideal gigas state, in that the individual linear dimensions of the tetraploid were not 1.26 ($\sqrt[3]{2}$) times those of the diploid leaf blade.

It is evident that, except for the number of cell divisions which contributed to leaf thickness, the cell-division activity from plane to plane and from tissue to tissue in the tetraploid differed from that of

the diploid. This difference existed also in the degree of cell enlargement from diameter to diameter and from tissue to tissue, since in these characteristics there was no evident simple relationship between tetraploid and diploid. Obviously, the relation of the growth rates of the respective leaf blades to the rates of increase in the number and size of their constituent cells was extremely variable in the different parts of the leaf blade.

Since the growth rate of the tetraploid leaf blade was slower and the initiation of maturation later, it is apparent that, with the same number of cells in the mature tetraploid and diploid leaves, the rate of cell division must have been correspondingly slower in the tetraploid.

The problem of the rate of increase in average cell size cannot be dealt with in this simple manner. It is evident, however, that the tetraploid cells reach a greater size before dividing than do the diploid cells; and since the growth rate of the tetraploid leaf blade as a whole was slower than that of the diploid, it is logical to assume that the rate of increase in the volume of the individual tetraploid cells is slower than that in the diploid cells.

If one accepts as valid the well-known concept that an optimum surface-volume relation is essential to normal growth, it may be inferred that the greater absolute size of the cell and nucleus in the tetraploid has had a major influence in reducing the rate of growth and the ultimate size of the organs attained by the tetraploid plant. Since the volume of the tetraploid cell increased as the cube of the linear dimensions of the diploid, and the area of its limiting membrane increased merely as the square of these same dimensions, as a result of chromosome doubling, some degree of metabolic unbalance might be expected during the development and differentiation of the tetraploid, especially in the more highly differentiated organs, such as the leaf blade. The concept is a familiar one and needs no further elaboration in the absence of a specific and detailed factual basis in our material; yet it cannot be ignored as an important physiological factor which may be of potential major significance in the economy of the polyploid plant and which may be held indirectly responsible, at least in part, for the more sluggish growth rate of the tetraploid leaf blade.

With the increasing functional specialization of the maturing tissues of the leaf blade, there is ample opportunity for many diverse factors to interact in a manner to account for the notable lack of uniformity between the quantitative behavior of the individual cells and of the leaf as a whole. This lack of uniformity in the form and cell relations of the mature leaf is in such striking contrast to the uniformity throughout the unspecialized shoot apex that the coincidence between functional specialization and lack of uniformity seems to assume a probable cause-effect relationship.

Genetic constitution may also play a major role in modifying the quantitative response to the mechanical increase in nuclear size, not only, as just suggested, within the individual genotype as ontogeny proceeds, but also from genotype to genotype. Certainly there is variation of a significant nature between cell size and organ dimensions from genetic stock to genetic stock, as indicated in tables 3, 4, and 5. It is clearly apparent that the cells of the same tissue or of the same leaf dimension in the tetraploid differ in the degree of their departure from the diploid condition, depending on the genotype in which doubling has

occurred. This fact may be interpreted as evidence that the ultimate effect of doubling the number of genes through doubling the number of chromosomes is not always arithmetic in terms of cell or organ volume at maturity. Yet the shoot apex, both in cell and organ size, has responded to tetraploidy almost exclusively by the doubling of volumes. The genic complement, or genome, of the cells is the same in the shoot apex as in the mature leaf, yet these two organs respond differently to chromosome doubling.

A relatively simple explanation of this situation would be to assume that different genes belonging to the same genome, operating at different stages in the life cycle and responding differently to a reduplication of their number, are responsible for the observed differences in the effect of chromosome doubling on the shoot apex and on the mature leaf blade. The genes controlling the size, shape, and cell-pattern relations of the shoot apex in maize responded in a similar manner to chromosome doubling, with the result that the tetraploid shoot apex was enlarged symmetrically and attained the ideal gigas state. But this was not true in the tetraploid shoot apices of *Vinca* studied by Cross and Johnson (*3*), in which chromosome doubling produced an increase in cell width but had no effect on other dimensions of the cells. However, the doubling of the genes concerned with the development of the pattern of the mature maize leaf blade resulted in responses that produced alterations in the symmetry of the pattern as it existed in the diploid plants, and the end result of these alterations was a tetraploid leaf pattern that had no simple arithmetical relation to the original diploid pattern.

With respect to the shoot apex in the genetic stocks that were studied in this investigation, the doubling of cell size and organ size in the tetraploid appeared to be correlated directly with the doubling of the number of chromosomes, and there were no departures from this condition that might be attributed to the nonuniform action of specific genes. However, in the mature leaf there was a conspicuous lack of uniformity in the response of the organ as a whole and of its constituent cells and tissues to chromosome doubling, both in different regions of the same leaf and in the leaves of different stocks. This suggests that certain genes belonging to particular genomes responded differently to chromosome doubling. The cumulative or quantitative action of certain genes, and its absence in others, would account for the observed differences between the various diploid and tetraploid maize stocks with respect to leaf morphology.

Similar differences in the response of certain genes to chromosome doubling presumably resulted in percentage increases in the carotenoid content of certain strains of tetraploid maize and percentage decreases in other strains analyzed by Randolph and Hand (*10*). Likewise, the decrease in stature and fertility that accompanies chromosome doubling in inbred strains of maize and the absence of these deleterious effects when the chromosomes of hybrid stocks are doubled, which was interpreted by Randolph (*8*) as an indication that heterozygosity per se may be an important cause of hybrid vigor, are explainable on a genic basis in terms of similar disharmonious types of quantitative gene action. Unfortunately, the genic analysis required to validate these suggested interpretations is not easy to obtain. The inheritance of such characters is presumably quantitative, and tetraploidy imposes further complications.

An interpretation of the morphological significance of these observations involves not so much a comparison of individual dimensions as the relation between dimensions, this being the basis of form. As already stated, the form of the tetraploid and diploid shoot apices was essentially the same during the period of initiation of the tenth leaf primordium (i. e., during plastochron 10), but the tetraploid was twice the volume of the diploid. The basal circumference of the tetraploid approximated the expected value of $\sqrt[3]{2}$ times that of the diploid. Throughout the development of the tenth leaf blade, its width for any given length was practically as much greater in the tetraploid than in the diploid as the tetraploid shoot apex was greater in basal circumference, and therefore as the tetraploid leaf primordium was greater in width. The original advantage in width given to the tetraploid leaf blade by its origin from a larger shoot apex was maintained throughout ontogeny.

An analogous situation was described by the writers (*1*) in the ontogeny of successive leaves of the diploid plant. The shoot apex in the ontogeny of the diploid became increasingly larger in each successive plastochron, and the leaf blade originating in each plastochron was as much wider at any given length than the preceding one as the shoot apex was greater in basal circumference than the preceding one; that is, in the ontogeny of the diploid there is an increase in size of leaf initial and this advantage is maintained throughout ontogeny. In the case of the ontogeny of the diploid, the increasing size of the shoot apex was due primarily to an increase in the number rather than in the size of the constituent cells. The comparison of the tetraploid and diploid shows that the leaf which is wider for any given length originates from the larger shoot apex. But in this case the shoot apex was larger because of larger cells and not because of more cells.

There was a change in the form of successive leaves in the ontogeny of the diploid, and there was a similar difference in the form of the tetraploid and diploid leaves. Both changes in form were correlated with a similar change in form of the shoot apex. But the shoot apex in the ontogeny of the diploid changed in form because of an increase in number of cells, whereas that of the tetraploid changed because of an increase in cell size. In neither can the change of form be attributed to a difference in the quality of the genome. In the ontogeny of the diploid the change is a characteristic expression of the diploid genome through its influence on rate of cell division in the shoot apex, on the one hand, and of rate of initiation of new leaf primordia on the other. Cells multiplied in number in the shoot apex more rapidly than leaf initials were set off in successive plastochrons, so that the shoot apex became increasingly larger and, therefore, the successive leaf initials also became larger. There was no basic difference in the manner in which growth in width was correlated with growth in length in the ontogeny of successive leaves in the diploid, the wider the leaf initial the wider the developing leaf, but this was obviously not due to a change in the genetic constitution of the plant. Similarly, there is no qualitative difference in the genetic constitution of the tetraploid and diploid plants.

The greater size of the tetraploid shoot apex can be traced directly to a mechanical increase in nuclear size due to the presence of twice as many chromosomes as are present in the diploid. This change did

not modify the number of cells in the shoot apex during the tenth plastochron. Therefore, the greater width for any given length of the tetraploid leaf blade can be considered a residual effect of changed nuclear size in the shoot apex. Thus we have two mechanisms, nongenetic in nature, that may result in changes in the form of the leaf blade. In each case the changes may be traced directly back to the gross size of the shoot apex. In one case the size of the shoot apex changes because of a change in the number rather than in the size of the constituent cells (ontogeny of $2n$); in the other case, because of a change in the size rather than in the number of cells ($4n$ vs. $2n$).

The changes in the developmental morphology of the leaf blade resulting from chromosome doubling are of such a nature at different periods or ontogeny that there is a basis for distinguishing between the direct effect of increasing nuclear volume on cell size and organ size and the indirect effect of doubling the number of specific genes. Earlier in the development of the tetraploid maize plant the mechanical effect of doubling the number of chromosomes seems the more important, and there is a residual effect of this which may be observed in subsequent development. Increasingly important in later development are the morphological effects evident in the departure of cells and of linear dimensions from the perfect gigas state. This may be attributed, in part at least, to doubling the number of genes. A gene complex that affects a given function (and thus the cell type in which the function is concentrated) may react differently in the doubled state from other gene complexes affecting other functions and, therefore, other tissues. This latter conclusion is based on the varying responses to tetraploidy noted in passing from one region to another in the mature leaf, as well as the varying responses noted in the same leaf region of different stocks.

SUMMARY

A comparative study was made of the shoot apex and tenth leaf in diploid and tetraploid maize, with special reference to the form and structure of the shoot apex during the time of initiation of the tenth leaf primordium (i. e., during the tenth plastochron), the development of the leaf, and the structure of the mature leaf.

The shoot apex of tetraploid plants during plastochron 10 had the same number and arrangement of cells as in the corresponding diploid plants, but the volume of the nuclei and cells was doubled. In consequence of the larger cell size, the tetraploid shoot apex also was correspondingly larger and the tenth leaf initial, which originates as a ridge of tissue that almost completely surrounds the base of the shoot apex, was correspondingly wider than in diploid plants.

The rate of increase in both width and length was slower for the tetraploid than for the diploid leaf blade. Also, growth in length and growth in width were so related that for any given increment in length there was a proportional increment in width; the tetraploid leaf initial was wider at the time of its origin, and this original difference in width for any given length was maintained throughout ontogeny.

At maturity there was a marked tendency in different strains for the tetraploid leaf to be of approximately the same length as the diploid, about 1.16 times as wide, and 1.35 times as thick. The volume of the tetraploid leaf at maturity was thus about 1.6 times

the volume of the mature diploid leaf. The total number of cells in the mature leaf blade was estimated to be about 143×10^6 in the tetraploid and about 154×10^6 in the diploid plants, the difference being of questionable significance. Average cell volume in the tetraploid was about 1.6 times that of the diploid, but detailed analysis of cell size from one tissue region to another indicated failure to maintain this relationship from region to region. The relationship between cell dimensions in the diploid and tetraploid leaf blade was extremely variable in the various tissues in the same region and in different regions of the leaf.

In the fully differentiated tissues of the mature leaf there was no consistent relation between the dimensions of the organ and its constituent cells, such as existed in the undifferentiated tissues of the shoot apex. This suggests that the cell may exert a more potent influence on organ pattern in relatively simple embryonic structures than it does in more highly specialized organs of the mature plant.

The departure of the mature leaf, with its fully differentiated cells and tissues and its marked segregation of functions, from the simple cell-organ relationship characterizing the relatively undifferentiated shoot apex was examined in relation to a number of different factors. Among these were included the rate of increase in cell number and size as related to growth rate, the possible influence of absolute cell size on rate of cell division, surface-volume relations of the cells, the development of functional specialization of the maturing tissues of the leaf, and the evidence of genetic modification of any tendency to maintain the initial effects of chromosome doubling throughout ontogeny.

The concept of an "ideal gigas state" is developed as an aid in the interpretation of the diverse effects of chromosome doubling. This state is attained when the volume of the various structures in the tetraploid, including cells, tissues, and organs, is double that of the diploid, but the relative dimensions of these same structures remain the same; that is, when the external form of the tetraploid structural unit is the same as that of the diploid but its volume is doubled and, as a consequence, the ratio between the surface area of the tetraploid and that of the diploid equals $\sqrt{2}$, or 1.41, and the ratio between the linear dimensions of the tetraploid and those of the diploid equals $\sqrt[3]{2}$, or 1.26. The ideal gigas state was attained in the shoot apex of tetraploid maize, but not in the gross structure and cellular components of the various tissues of the mature leaf.

The significance of doubling the number of chromosomes vs. doubling the number of specific genes in relation to their possible influence on the morphology of tetraploid and diploid maize is discussed. Apparently the relative importance of these two factors differed at different stages in ontogeny. Early in the ontogeny of the shoot apex and leaf primordium, doubling the number of chromosomes resulted in a uniform increase in cell size and organ size with no evidence of differential gene action on individual dimensions. Later in ontogeny, as indicated by the form and structure of the mature leaf, there was abundant evidence of differential gene action. The form of the tetraploid leaf blade was consistently different from that of the comparable diploid in that it was wider and thicker but not longer; and there were also significant form differences between tetraploid stocks of different genotypic constitution and the comparable diploid stocks.

It is suggested that deviations from the ideal gigas state, which were prevalent in the mature leaves and other organs of tetraploid maize, may be due to the cumulative or additive effect of certain genes but not of others, and that the diverse results of chromosome doubling in different stocks of maize, as well as in other organisms, may be due primarily to differences in genotypic constitution.

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DAMPING-OFF IN BROADLEAF NURSERIES OF THE GREAT PLAINS REGION[1]

By ERNEST WRIGHT[2]

Associate pathologist, Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture

INTRODUCTION

At present there are relatively few pertinent data, in either American or foreign literature, on the etiology of damping-off of broadleaf seedlings, partly because seedling diseases in broadleaf nurseries have been of less importance than those in coniferous nurseries (*1*).[3] However, since the establishment of large Federal nurseries for the production of deciduous stock in 1935, damping-off has assumed considerable importance in the culture of some broadleaf species.

This paper gives the results of some recent field and laboratory studies on the damping-off of broadleaf seedlings in Federal nurseries of the Great Plains region.

LOCATION AND SOIL AND CLIMATIC CONDITIONS OF NURSERIES

To understand fully the damping-off problem involved, it is desirable to consider the location of the nurseries in relation to the soil and climatic conditions of the Great Plains region.

In 1940 there were 17 Federal broadleaf nurseries in the Great Plains, located between latitude 34° and 47° north and longitude 95° and 103° west. Four of the nurseries were maintained by the Soil Conservation Service; the others were operated by the Prairie States Forestry Project. Figure 1 shows the approximate location of these nurseries.

Along the 100th meridian the average annual precipitation in the northern Great Plains is 16–18 inches, whereas in the Panhandle of Texas it is 22–24 inches.[4] Variations in precipitation between northern and southern nurseries were, however, fairly well equalized by higher evaporation losses in the South. During the growing season the average maximum temperature in northern nurseries was 84° F.,

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[2] This study was initiated under the direction of Dr. George L. Peltier and continued under the supervision of Dr. Robert W. Goss, of the University of Nebraska. The writer gratefully acknowledges this guidance and also expresses his thanks to the following individuals for valuable advice, assistance, and suggestions: Dr. K. F. Baker, Dr. Carl Hartley, Dr. W. J. Himmel, Dr. J. E. Livingston, Dr. K. S. Quisenberry, and Dr. Leva B. Walker. Assistance of the personnel of the Prairie States Forestry Project, Forest Service, and the Soil Conservation Service, of the U. S. Department of Agriculture, is also acknowledged. Greenhouse and laboratory equipment was generously provided by the Department of Plant Pathology of the University of Nebraska.

[3] Italic numbers in parentheses refer to Literature Cited, p. 93.

[4] Data from U. S. Weather Bureau, 40-year period, 1895–1934.

whereas in the South it rose to 94°.[5] Wide temperature fluctuations are characteristic of both the southern and northern nurseries during the spring (April to June), when damping-off is most prevalent.

Most of the Federal nurseries in this region were located on the zonal soil group known as the Chernozem, on which the native vegeta-

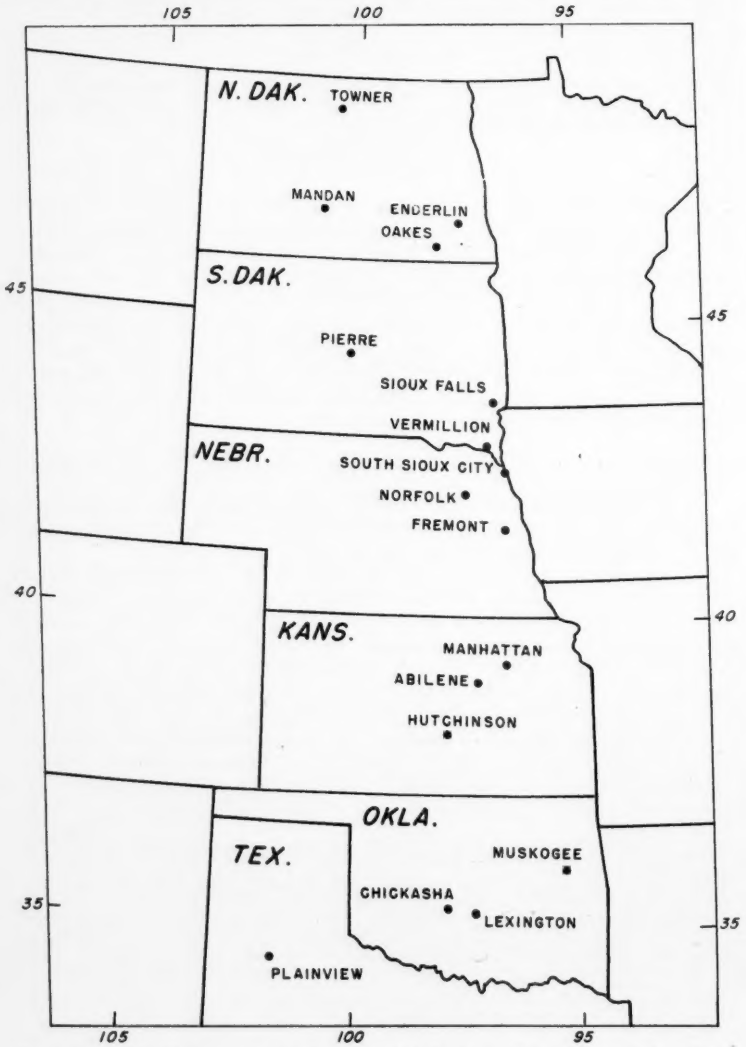

FIGURE 1.—Outline map, showing the distribution of nurseries of the Prairie States Forestry Project and Soil Conservation Service in the Great Plains region in 1940.

[5] Data from U. S. Weather Bureau, 30-year period, 1904–33.

tion was tall grass prairie and the soil developmental process was by calcification. Two of the nurseries, one at Mandan, N. Dak., and the other at Pierre, S. Dak., were within the Chestnut zonal soil group, which was also developed by calcification but on which the native vegetation was short grass prairie. Nurseries now or formerly located at Muskogee, Tecumseh, Oklahoma City, and Lexington, Okla.; Winfield, Kans.; and Fremont, Nebr., belong in the Prairie zonal soil group, which developed by calcification with some podzolization and originally had tall grass native vegetation (*18*).

The developmental process by calcification common to the soils of this region is characterized by such restricted leaching that the carbonates of calcium (lime) and magnesium accumulated somewhere in the lower or upper horizon of the soil (*8*). In reaction, therefore, the soils are characteristically neutral or slightly acid, frequently alkaline, but never strongly acid.

Climatic conditions and soil formations throughout the Great Plains region, therefore, are unusually uniform for such a great extent of latitude. The location of many of the broadleaf nurseries on sandy sites has further equalized natural soil differences. This uniformity tends to simplify the damping-off problem and makes it possible to compare losses directly between nurseries throughout the area.

CULTURAL PRACTICES IN PRODUCTION OF DECIDUOUS SEEDLINGS

Federal nurseries producing stock of broadleaf species in the Great Plains differ considerably from coniferous nurseries in this region. The deciduous seedlings are grown in drills or rows in open fields without benefit of artificial shade, frequent watering, or protection by seedbed frames (fig. 2). The general procedure is to sow the seed in drills,

FIGURE 2.—Prairie States Forestry Project nursery at Fremont, Nebr., showing seedlings of broadleaf species grown in open, unprotected rows in the Great Plains region. Photograph from Forest Service, U. S. Department of Agriculture.

which are later ridged to prevent excessive drying of the soil in contact with the seed. As soon as the seeds begin to germinate the ridge is removed by hand raking. If germination is delayed by lack of precipitation, the sowings are irrigated by running a small stream of water between the rows. In a few nurseries water is supplied through elevated pipes, a method generally referred to as "overhead irrigation." After the seedlings emerge, they are irrigated from time to time, depending on the weather and the judgment of the nurserymen.

Density of sowing is mainly influenced by the viability of the seed and the probability that the seedlings will emerge and survive. The general practice is to sow the seed densely enough to obtain at least 16 seedlings per lineal foot of row. During the stage when the seedlings are most susceptible to damping-off they are on an average about one-half inch apart. Depth of sowing varies according to species.

The rows of seedlings are spaced from 21 to 30 inches apart, to permit machine cultivation and weeding in a manner similar to that used for truck crops. With the density and spacing described, there are from 100,000 to 250,000 seedlings grown per acre.

DAMPING-OFF OF SEEDLINGS OF BROADLEAF SPECIES

DESCRIPTION OF THE DISEASE

Two types of damping-off are common among broadleaf species in Great Plains nurseries, namely preemergence and postemergence damping-off. Preemergence damping-off, which results in the destruction of the seed or newly germinated seedling, prevents emergence and leaves vacant spots in the drills. This type of loss has frequently and erroneously been attributed to poor seed. Postemergence damping-off takes place after the seedlings emerge and may cause considerable loss until the plants are 3 to 4 weeks old. Sore shin,[6] which may be regarded as a phase of postemergence infection, stunts older seedlings but is not necessarily fatal and is quite common.

Damping-off caused by top infection has occurred only rarely in this region and is not regarded as an important problem during seasons of above-normal precipitation except in nurseries located on heavy soil. Late root rots caused by damping-off parasites are also of relatively little importance.

For broadleaf species, postemergence damping-off is usually atypical in that the infected seedlings do not become flaccid and fall prostrate on the soil. Instead, they commonly remain in an upright position, gradually wilt, break off, and finally are blown away. This type of loss has no doubt led to the prevailing opinion that postemergence damping-off of broadleaf species is rare. Only careful daily counts will prove otherwise, since such losses during 24 hours are seldom large but over a cumulative period of 10 days not uncommonly amount to 20 to 35 percent of the stand.

Postemergence damping-off of seedlings of broadleaf species is most severe when the seedlings are in the cotyledon stage. The heaviest

[6] The term "sore shin" appears to have been used originally by Dr. George F. Atkinson in 1892 for describing a disease symptom of cotton in Alabama.

losses occur at about the time the cotyledons cease to function and the true leaves are forming. As soon as the first true leaves are fully developed the critical damping-off period is past. At high soil temperatures the period of susceptibility may decrease, but losses are frequently greater, owing to increased activity of the fungus parasites.

Diseased seedlings 2 weeks to 1 month old commonly show a reddish-brown lesion on the stem near the ground line (fig. 3). This

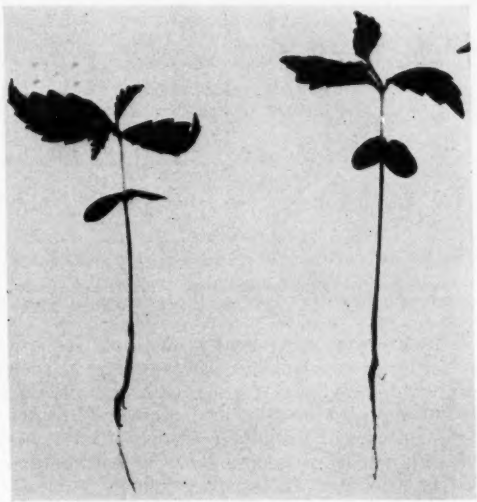

FIGURE 3.—Sore shin infection of American elm seedlings about 5 weeks old. Infection shows at base of seedlings. Note development of second pair of true leaves. These seedlings did not fall over and lie prostrate on the soil but continued to live.

condition, known as sore shin, is, as already mentioned, seldom fatal, but the growth of infected seedlings is reduced. These stunted plants occasionally do not develop to usable size in one season, particularly in northern nurseries.

SUSCEPTIBILITY OF SEEDLINGS IN THE FIELD

Field observations, together with greenhouse tests, indicated that not all deciduous seedlings are subject to damping-off. Table 1 gives the damping-off loss in percentage for several important broadleaf species at a number of different nurseries for the 3-year period 1936–38.

The following are the most important broadleaf species susceptible to damping-off in the field:

American elm	*Ulmus americana* L.
Siberian elm	*Ulmus pumila* L.
Chinese elm	*Ulmus parvifolia* Jacq.
Black locust	*Robinia pseudoacacia* L.
Russian mulberry	*Morus alba tatarica* (L.) Ser.
Desertwillow	*Chilopsis linearis* (Cav.) Sweet.
Russian-olive	*Elaeagnus angustifolia* L.

TABLE 1.—*Calculated damping-off losses in Great Plains nurseries, based on plot counts*

Year	Locality	Species	Plot counts [1]	Damping-off
			Number	*Percent* [2]
1936	Rayland, Tex.	American elm	20	15
	do	Black locust	10	60
	Tecumseh, Okla.	Siberian elm	10	60
	Noble, Okla.	do	10	30
	Plainview, Tex.	do	10	35
	do	Black locust	10	60
1937	Mangum, Okla.	American elm	10	40
	do	Siberian elm	10	10
	Noble, Okla.	do	12	20
	Manhattan, Kans.	do	35	20
	Fremont, Nebr.	do	120	35
	Enderlin, N. Dak.	American elm	30	20
	Plainview, Tex.	Siberian elm	25	10
	do	Desertwillow	25	10
	Noble, Okla.	do	20	35
1938	Hutchinson, Kans.	American elm	20	35
	do	Siberian elm	20	20
	Manhattan, Kans.	American elm	30	15
	Norfolk, Nebr.	Siberian elm	33	15

[1] Counts made in 1936 and 1937 were based on regularly distributed 1-foot quadrats, but in 1938 these were 3 feet long.

[2] Percentage of damping-off was determined by comparisons with zinc oxide treated seed; therefore the calculated losses are considerably lower than if based on stands growing under sterile conditions.

Damping-off losses were also heavy at times for other broadleaf species of less importance, such as Osage-orange (*Maclura pomifera* (Raf.) Schneid.), Siberian pea (*Caragana arborescens* Lam.), silverberry (*Shepherdia argentea* Nutt.), and dogwood (*Cornus* spp.).

A number of seedlings of broadleaf species showed high resistance to damping-off, and control measures have been unnecessary for such species. A list of the resistant species follows.

Green ash	*Fraxinus pennsylvanica* var. *lanceolata* (Borkh.) Sarg.
Northern catalpa	*Catalpa speciosa* Warder.
Common hackberry	*Celtis occidentalis* L.
Netleaf hackberry	*Celtis reticulata* Torr.
Common honeylocust	*Gleditsia triacanthos* L.
Bur oak	*Quercus macrocarpa* Michaux.
Common chokecherry	*Prunus virginiana* L.

Other, less important broadleaf species rarely seriously affected by damping-off fungi are: Kentucky coffeetree (*Gymnocladus dioicus* (L.) K. Koch), walnut (*Juglans* spp.), western soapberry (*Sapindus drummondii* Hook. and Arn.), and tree of heaven (*Ailanthus altissima* (Miller) Swingle).

DAMPING-OFF HAZARD IN NURSERY SOILS

In greenhouse tests, soil was taken from each nursery in 1935 and placed in 4-inch clay pots. Three pots, filled with the same unsterilized soil from 15 different nurseries, were each sown with 50 seeds of Siberian elm. One pot of each set was placed in a section of the greenhouse where air temperatures ranged from 55° to 70° F.; a second pot was held at temperatures ranging from 60° to 85°, and a third pot at temperatures of 65° to 100°. Moisture conditions were kept as nearly comparable as possible during the tests. Postemergence damping-off counts and isolations were made daily. Preemergence losses were verified by examinations at the end of the tests. Sterile sand

checks, kept under similar conditions, made it possible to determine the losses on a percentage basis.

The nursery soils were grouped according to the severity of damping-off as shown in these tests. Since the tests were of limited extent, only an approximate arrangement was attempted. The results are summarized in table 2.

Hartley (*6*) and Jackson (*7*) found that incidence of damping-off of coniferous species is directly correlated with increase in pH values.

TABLE 2.—*Damping-off losses of Siberian elm grown in untreated nursery soils under greenhouse conditions*

Locality	Soil texture	Soil acidity	
		pH [1]	Depth
			Inches
SOILS WITH LIGHT DAMPING-OFF (LESS THAN 10 PERCENT)			
Enid, Okla.	Fine sandy loam	6.0	
Noble, Okla.	Very fine sandy loam (containing lime).	8.8	
SOILS WITH MODERATE DAMPING-OFF (10 TO 30 PERCENT)			
Enderlin, N. Dak.	Fine sandy loam	7.2–7.5	
Valley City, N. Dak.	Silty clay loam	6.9–7.5	
Baltic, S. Dak.	do	7.0–7.5	
Arlington, Nebr.	do	5.8	1
		6.0	6
Fremont, Nebr.	Silt loam	6.3	1
		6.3	6
Abilene, Kans.	do	6.0	1
		6.4	6
Winfield, Kans.	do	6.4	1
Oklahoma City, Okla.	Silt clay loam	5.8–6.4	
Chillicothe, Tex.	Fine sandy loam	7.2	1
		7.0	6
Rayland, Tex.	Very fine sandy loam	6.7	1
SOILS WITH HEAVY DAMPING-OFF (MORE THAN 50 PERCENT)			
Brookings, S. Dak.	Silty clay loam	7.8	
McPherson, Kans.	do	6.0	1
		6.0	1
Tecumseh, Okla.	Silt to clay loam	6.0	1
		6.5	6

[1] Colorimetric determinations.

In the tests conducted by the writer there does not appear to be a definite correlation between soil acidity and damping-off of Siberian elm. There was some indication in greenhouse tests, however, that damping-off losses of broadleaf species were least on sandy soils and heaviest on clay loam.

DAMPING-OFF FUNGI

ISOLATION TECHNIQUE

Diseased seedlings were collected daily from test pots in the greenhouse, and isolations of damping-off fungi were made. The technique used throughout this study was to remove the infected seedlings in toto and wash the roots in running water to remove the soil. The roots and root crown were then cut off with flame-sterilized scissors. A small section, ½ to 1 cm. long, was next cut from the infected part

so as to include sound as well as obviously diseased tissue. These small sections were immersed in mercuric chloride (1:2,000) for 1 minute, rinsed thoroughly in sterile water, then placed between sterile filter paper for at least 5 minutes to remove excess water, and finally inserted into Petri dishes containing malt agar (pH 5.5). The cultures were incubated at 22° to 25° C. For later examination, transfers were made to malt agar in test-tube slants.

HOSTS AND LOCALITIES

In 1921 Hartley (*6*) listed several deciduous species reported to be infected with damping-off fungi. In a later paper Wright [7] summarized the literature up to 1937 and reported some additional broadleaf species affected with damping-off. Crandall [8] also added other hosts to the list. Since 1937 the writer has made many other isolations from diseased seedlings of broadleaf species. The results of these isolations are given by species and locality in table 3.

Pythium ultimum Trow was [9] the only species of *Pythium* commonly isolated from seedlings of broadleaf species showing symptoms of damping-off. No noticeable variations have been observed among the many *P. ultimum* isolates obtained in culture, either when coming from different hosts or from the same host growing in different soils.

TABLE 3.—*Isolations obtained from deciduous seedlings grown in Great Plains nursery soils*

Host	*Rhizoctonia solani*[1]	*Pythium ultimum*[2]	Locality	Host	*Rhizoctonia solani*[1]	*Pythium ultimum*[2]	Locality
American elm (*Ulmus americana*).	-----	×	Arlington, Nebr.	Desertwillow (*Chilopsis linearis*).	×	×	Lincoln, Nebr.
	×	-----	Brookings, S. Dak.		×	-----	Rayland, Tex.
	×	-----	Fremont, Nebr.	Green ash (*Fraxinus pennsylvanica* var. *lanceolata*).	×	-----	Enid, Okla.
	×	×	Lincoln, Nebr.				
	×	-----	Manhattan, Kans.				
	-----	×	Muskogee, Okla.				
	×	-----	Oklahoma City, Okla.	Siberian pea (*Caragana arborescens*).	×	-----	Mandan, N. Dak.
	×	-----	Rayland, Tex.				
	×	-----	Winfield, Kans.	Silver maple (*Acer saccharinum* L.)	×	-----	Enid, Okla.
Black locust (*Robinia pseudoacacia*).	×	×	Lincoln, Nebr.				
	-----	×	Manhattan, Kans.	Northern catalpa (*Catalpa speciosa*).	-----	×	Abilene, Kans.
	×	×	Rayland, Tex.		×	-----	Arlington, Nebr.
Siberian elm (*Ulmus pumila*).	-----	×	Abilene, Kans.		-----	×	Winfield, Kans.
	×	-----	Arlington, Nebr.				
	-----	×	Baltic, S. Dak.				
	×	-----	Brookings, S. Dak.				
	×	-----	Chillicothe, Tex.				
	×	-----	Lexington, Okla.				
	×	×	Lincoln, Nebr.				
	×	-----	Manhattan, Kans.				
	×	×	Mandan, N. Dak.				
	-----	×	Noble, Okla.				
	-----	×	Oklahoma City, Okla.				
	×	-----	Rayland, Tex.				
	×	-----	Salina, Kans.				
	×	×	Sioux Falls, S. Dak.				
	×	×	Tecumseh, Okla.				
	×	×	Winfield, Kans.				

[1] All *R. solani* isolates were obtained from infected roots of young seedlings damping-off except that from green ash, which was suffering from top infection, and Siberian pea, 1 year old, showing late root rot.
[2] All *P. ultimum* isolates came from infected roots of young seedlings.

[7] WRIGHT, E. DECIDUOUS-SEEDLING DISEASES IN MIDWEST NURSERIES. U. S. Bur. Plant Indus. P Dis. Rptr. 21: 80-81. 1937. [Processed.]
[8] CRANDALL, B. S. RHIZOCTONIA ON TREE SEEDLINGS. U. S. Bur. Plant Indus. Plant Dis. Rptr. 21: 8 1937. [Processed.]
[9] Identification was verified through the courtesy of Dr. John T. Middleton, formerly of the Department of Botany, University of Missouri.

Variations were common among *Rhizoctonia solani* isolates obtained from different species, even when the hosts were grown in the same soil; these variations were most noticeable when the isolates were all grown on the same media and held under similar conditions. Macroscopic appearance has proved to be more dependable than microscopic measurements for distinguishing *R. solani* isolates. A similar conclusion was reached by Matz (*11*) and Briton-Jones (*2*).

All isolates obtained during this study are regarded as strains of *Rhizoctonia solani* Kühn. Cultural variations did not appear to be constant enough to justify separating the *Rhizoctonia* isolates into species as was done by Matz (*11*) and Simon-Thomas (*15*) (fig. 4). The *Corticium*, or perfect stage, was not observed in culture during the course of these investigations.

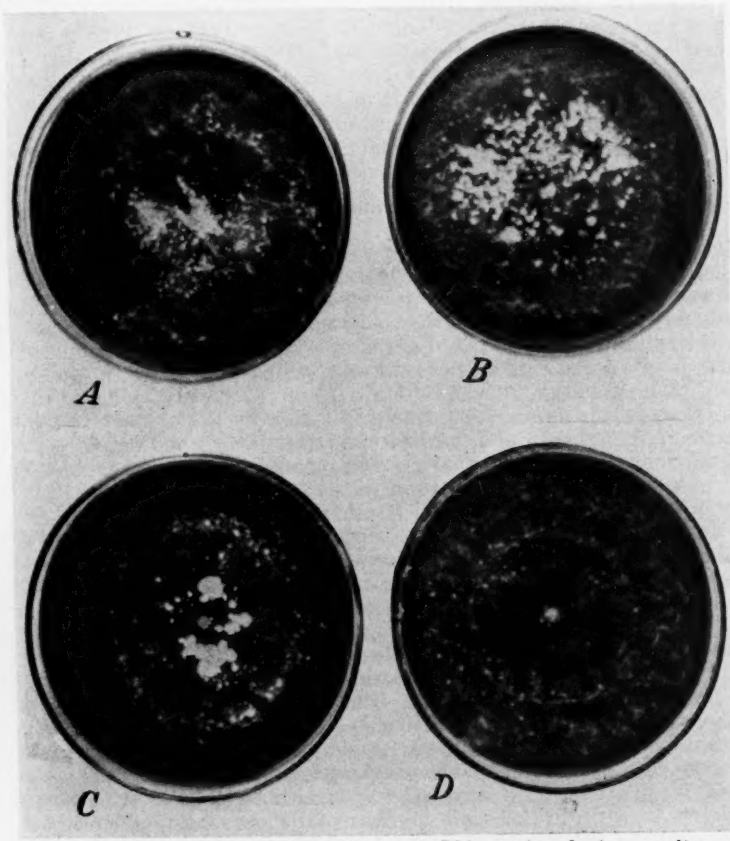

FIGURE 4.—Typical 2-week-old cultures of *Rhizoctonia solani* on malt agar. These isolations were obtained from seedlings of (*A*) American elm, (*B*) black locust, (*C*) Siberian elm, and (*D*) desertwillow, grown in the same Marshall silt loam soil. The cottony appearance of the sclerotia was characteristic of these *R. solani* isolates, but their abundance and distribution within the individual cultures were not always constant.

The isolations made during these studies indicated that American and Siberian elm seedlings are both commonly infected with *Rhizoctonia solani* and *Pythium ultimum*. Black locust appeared to be more frequently attacked by *P. ultimum*, and desertwillow by *R. solani*. Isolations for other broadleaf species were not numerous enough to warrant any conclusion as to which parasites were most prevalent, but *R. solani* and *P. ultimum* were most frequently isolated. *Fusarium* spp. also were frequently isolated from seedlings of broadleaf species that showed damping-off symptoms, and an occasional *Phytophthora* was obtained in culture. No attempt was made to identify the different isolates of *Fusarium*, since, judging by their prevalence, they were of secondary importance. All the *Fusarium* species obtained appeared to belong in the section *Elegans*.

It was not uncommon to obtain more than one organism in culture from the same diseased seedling. Frequently bacteria were present together with fungi, especially with *Pythium*. Occasionally *P. ultimum* and *Rhizoctonia solani* were isolated from the same seedling. During these studies the sclerotia of *R. solani*, such as are commonly found on potato tubers, were never observed on any of the broadleaf species. This may be due to the fact that after infection the seedlings soon decay.

PATHOGENICITY TESTS THROUGH SOIL INOCULATION

Matsumoto (*10*), Richter (*13*), and Simon-Thomas (*15*) have concluded that *Rhizoctonia solani* is more virulent on the host from which it was originally isolated than it is on a different host species. Gratz (*5*) and Wellman (*19*) found that *R. solani* from potatoes did not cause wire stem of cabbage, while Lauritzen (*9*) and Tervet (*16*) reported that potato isolates of this fungus were nonpathogenic to turnip and flax. The investigations of Sanford (*14*) and Garrett (*4*) showed that soil-inoculation tests are influenced not only by the character of the inoculum but also by soil sterilization. Hartley (*6*) had found earlier that inoculations with *Pythium debaryanum* Hesse[10] on unheated soils were much less destructive than on heated soils, and Sanford (*14*) found the opposite to be true of *R. solani*.

In the pathogenicity tests reported here an attempt was made to take these various factors into consideration, and a number of tests were made in both sterilized and unsterilized soil. In making soil inoculations for pathogenicity tests, about $\frac{1}{10}$ of a 90-mm. malt-agar Petri-dish culture was worked into the surface soil of each pot. Inoculations were made in both sterilized and unsterilized soil. Several days after inoculation, the seeds of broadleaf species were sown.

Early in these tests isolates of *Rhizoctonia* from American and Siberian elms (fig. 4) were used in a small series of cross inoculations in steam-sterilized soil. One-month-old cultures containing well-developed sclerotia were used. Eight 4-inch clay pots for each series were inoculated, and 3 days after inoculation the seeds were sown. Seeds were sown in the same number of pots containing sterilized, uninoculated soil, to determine seed viability as a basis for estimating preemergence losses. The results of these tests are presented in table 4.

[10] Hartley later expressed the opinion that the identification of the damping-off *Pythium* as *P. debaryanum* was probably incorrect and that he was working with *P. ultimum*.

TABLE 4.—*Pathogenicity tests of 2 Rhizoctonia solani strains introduced into sterilized loam*

[Air temperature, 60°–80°F.; duration of tests, 30 days]

Crop [1]	Total emergence		Preemergence loss based on checks	Postemergence loss based on stand in inoculated pots	Final stand based on checks
	Check	Inoculated			
AMERICAN ELM RHIZOCTONIA					
	Number	*Number*	*Percent*	*Percent*	*Percent*
Ponderosa pine	320	0	100		
Siberian elm	268	0	100		
Wheat (winter)	392	321	18	97	2
Alfalfa (Grimm)	356	128	64	88	5
Corn (Hogue Yellow Dent)	194	194	0	0	100
SIBERIAN ELM RHIZOCTONIA					
Ponderosa pine	320	135	58	10	38
Siberian elm	268	43	84	40	9
Wheat (winter)	392	314	20	57	49
Alfalfa (Grimm)	356	331	7	0	93
Corn (Hogue Yellow Dent)	194	183	6	0	94

[1] 400 seeds were used for all species except corn, for which 200 seeds were used. 8 pots were used for each test. Inoculations were made 3 days before sowing.

It is apparent from these cross-inoculations that the *Rhizoctonia solani* strains from both American and Siberian elms are pathogenic to a number of crops. However, the *R. solani* isolate from American elm proved somewhat more virulent than that from Siberian elm. This was particularly evident on ponderosa pine and Siberian elm. These results show that *R. solani* can cause heavy preemergence losses. The two *R. solani* strains tested, however, caused practically no preemergence loss for corn and very little for wheat. Postemergence infection of these two crops was nevertheless heavy. The wheat exhibited true postemergence damping-off symptoms by falling prostrate to the soil, but the corn showed only brownish infection spots, or sore shin, near the ground line. The infection spots were overgrown by the end of the tests, and there was no damping-off.

In a larger series of soil inoculations the pathogenicity of both *Pythium ultimum* and several strains of *Rhizoctonia solani* was tested on four broadleaf hosts. Marshall silt loam contained in 4-inch clay pots was inoculated with a week-old malt-agar culture. Twenty pots of steam-sterilized and unsterilized soil constituted each inoculation series, and the same number of pots containing uninoculated sterilized and unsterilized soil were set up as checks. All pots were sown with 100 seeds. The percentage of preemergence and postemergence damping-off occurring in these tests is given in table 5.

The general trend of the preemergence losses after inoculation with *Rhizoctonia solani* was greater in unsterilized than in sterilized soil, whereas the occurrence of postemergence damping-off was greater in sterilized soil. *Pythium ultimum* caused greater preemergence losses, except for desertwillow, in sterilized soil, in which, accordingly, less postemergence damping-off occurred. These tests did not show that the *R. solani* strains tested were most virulent to the host from which

TABLE 5.—*Effect of cross-inoculations in soil with Rhizoctonia solani and Pythium ultimum originally isolated from seedlings of broadleaf species growing in the same soil*

[5 pots of 100 seeds each]

STERILIZED SOIL [1]

Fungus inoculated into soil, and host source	American elm				Siberian elm				Black locust				Desertwillow			
	Preemergence loss		Postemergence loss		Preemergence loss		Postemergence loss		Preemergence loss		Postemergence loss		Preemergence loss		Postemergence loss	
	Mean	Standard error	Mean	Standard error	Mean	Standard error	Mean	Standard error	Mean	Standard error	Mean	Standard error	Mean	Standard error	Mean	Standard error
Rhizoctonia solani from—	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
American elm	39	±4.9	37	±5.4	57	±3.8	22	±4.8	47	±4.9	28	±2.0	54	±6.2	24	±1.7
Siberian elm	25	±6.8	48	±5.9	63	±2.8	17	±2.2	52	±3.6	27	±1.4	53	±6.2	28	±2.2
Black locust	25	±2.7	21	±5.9	39	±1.7	11	±5.5	28	±3.5	21	±2.0	41	±2.6	11	±.5
Desertwillow	65	±2.3	10	±3.3	64	±2.3	11	±2.8	62	±2.7	13	±3.3	71	±.21	4	±2.2
Pythium ultimum from—																
Siberian elm	40	±8.1	21	±5.2	40	±8.3	19	±2.8	38	±3.1	12	±2.0	68	±4.3	4	±2.0
Uninoculated soil	26	±9.9	29	±3.1	17	±6.2	18	±5.1	4	±2.3	18	±5.4	38	±1.2	18	±4.3
UNSTERILIZED SOIL																
Rhizoctonia solani from—																
American elm	64	±5.9	9	±2.6	74	±2.1	5	±0.18	69	±1.6	10	±3.3	60	±2.4	15	±2.0
Siberian elm	75	±1.6	5	±2.2	81	±.1	1	±.70	79	±1.9	5	±2.0	70	±2.8	11	±1.7
Black locust	38	±5.8	10	±2.0	43	±2.9	7	±1.4	34	±3.9	14	±.2	29	±7.5	12	±3.3
Desertwillow	64	±3.5	7	±5.0	66	±3.9	10	±2.2	53	±4.3	18	±1.7	55	±3.9	20	±3.7
Pythium ultimum from—																
Siberian elm	62	±2.9	9	±1.4	78	±.1	2	±.30	36	±4.2	9	±3.4	56	±3.5	11	±4.4
Uninoculated soil	63	±2.5	7	±1.4	77	±2.2	1	±.60	29	±4.1	13	±3.8	54	±6.4	18	±4.1

[1] Marshall silt loam soil steam-sterilized 1 hour at 5 pounds' pressure.

each was originally isolated. The question naturally arises as to why these results do not agree with the findings of Matsumoto (*10*), Richter (*13*), and Simon-Thomas (*15*). There are several possible explanations for this. The most probable is that the seedlings of the broadleaf species tested were all quite similar and did not show marked anatomical differences such as are displayed by agricultural crops, e. g., potatoes and sugar beets. It may also be significant that the isolates comprising the inocula were all obtained from diseased seedlings grown in the same soil. In the literature this point has not always been made clear, since the emphasis has been placed on the host rather than on both host and soil type. When these points are considered, it is more apparent why these tests do not show clear-cut variations in pathogenicity between isolates.

Of the broadleaf species used, black locust showed the greatest resistance to damping-off by both *Rhizoctonia solani* and *Pythium ultimum*. This reaction applied to the *R. solani* strain from black locust as well as to the isolates from the other hosts. It is known that these are atypical reactions for black locust, and it is probable that this apparent resistance was actually a partial escape from infection. Because of prompt seed germination and vigorous growth, the black locust seedlings appear to have passed the stage of greatest susceptibility before the soil inoculations became completely effective.

When black locust seedlings attacked by *Rhizoctonia solani* were examined soon after infection, it was found that discoloration and decay usually occurred first in the region of the root crown and that the tip of the root was destroyed last. Davis (*3*) described similar symptoms for longleaf pine seedlings infected with *Rhizoctonia solani*. Typical *Pythium ultimum* infection, however, early caused a decay of the tip of the root, and the root crown was not attacked until later. Hence the progress of infection for these two parasites was commonly directly opposite. Figure 5 shows these two types of

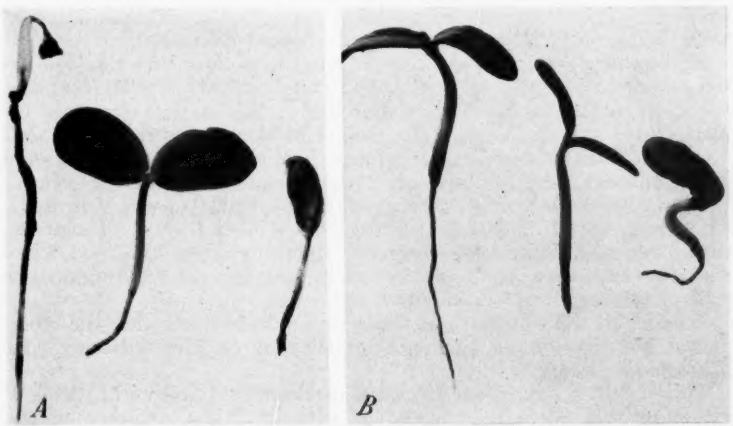

FIGURE 5.—Early stages of infection of black locust seedlings: *A, Rhizoctonia solani,* showing progress of decay from root crown downward toward root tip; *B, Pythium ultimum,* showing progress of decay from root tip upward toward root crown.

infection on black locust seedlings. Since the sterilized, uninoculated soil checks in the previous test also suffered from damping-off, a brief summary of the isolations obtained from diseased seedlings is presented in table 6.

TABLE 6.—*Summary of isolations obtained from damped-off seedlings in test presented in table 5*

Soil treatment and fungus used in inoculation	Isolate					
	R. solani		*P. ultimum*		Unclassified[1]	
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Sterilized soil:						
Rhizoctonia solani	107	68	5	3	46	29
Pythium ultimum	0		23	60	16	40
Checks	0		19	100	0	
Unsterilized soil:						
Rhizoctonia solani	68	58	18	15	32	27
Pythium ultimum	0		16	70	7	30
Checks	0		11	44	14	56

[1] Bacteria, other fungi, and blanks.

The isolations obtained from damped-off seedlings showed that steam-sterilized, uninoculated soil was contaminated with *Pythium ultimum*. Contamination probably came from adjacent inoculated pots through splashing of water. There were also some *P. ultimum* contaminations in the sterilized soil inoculated with *Rhizoctonia solani*. There were, however, no contaminations of *R. solani* in any of the series not inoculated with this fungus.

Regardless of the lack of variation in virulence between isolates or accidental contaminations, these tests show that *Pythium ultimum* and the *Rhizoctonia solani* strains tested are definitely pathogenic to young seedlings of American and Siberian elms, black locust, and desertwillow, as well as to several agronomic hosts. This comparatively wide range of pathogenicity further justifies designating these isolates of *Rhizoctonia* as strains rather than species. This is in accord with the view of Peltier (*12*), Tilford (*17*), and Wiant (*20*).

Preliminary tests with seedlings of these same four broadleaf species were made by inoculating sterilized and unsterilized soil with *Fusarium* sp. The method of inoculation used was similar to that employed for *Rhizoctonia* and *Pythium*. The results of these tests indicated that *Fusarium* is capable of causing fairly heavy preemergence losses in sterilized inoculated soil but very little in unsterilized inoculated soil. However, postemergence damping-off in sterilized soil was very light, whereas in unsterilized soil such losses were fairly heavy. *Fusarium*, therefore, may cause postemergence damping-off in the field when climatic conditions are favorable and in such instances may influence control measures.

A series of soil inoculations was also made to determine the effect of soil sterilization on the pathogenicity of *Pythium ultimum* and *Rhizoctonia solani*.

Thirty 7-inch glazed-earthenware crocks were filled with Marshall silt loam (pH 5.5–6.0) collected at Lincoln, Nebr. Fifteen of the crocks were sterilized for 3 hours at 20 pounds' pressure. The sterilized soil in 5 crocks was inoculated with *Pythium ultimum*, and the sterilized soil in 5 other crocks was inoculated with *Rhizoctonia solani*,

leaving 5 crocks of soil uninoculated as checks. Two-week-old inoculum was used. Immediately after inoculation, each of the 30 crocks was sown with 100 seeds of Siberian elm. The entire lot was then placed in cases with air temperature held at 75° F. Five days later the soil first inoculated with *P. ultimum* was inoculated with *R. solani*, and that first inoculated with *R. solani* was inoculated with *P. ultimum*. Damping-off counts and isolations were made periodically. The results of this series of tests are presented in table 7.

TABLE 7.—*Results of sowing Siberian elm seed in soil inoculated with both Pythium ultimum and Rhizoctonia solani* [1]

Soil treatment	Total emergence	Preemergence loss based on noninoculated pots	Postemergence damping-off based on stand	Final stand based on noninoculated pots
Pythium-inoculated soil plus *R. solani* 5 days later:	*Number*	*Percent*	*Percent*	*Percent*
Sterilized soil	18	95	61	2
Unsterilized soil	150	36	81	10
Rhizoctonia-inoculated soil plus *P. ultimum* 5 days later:				
Sterilized soil	178	49	80	11
Unsterilized soil	76	68	47	18
Uninoculated soil (check):				
Sterilized soil	348	0	0	100
Unsterilized soil	236	32	28	48

[1] 5 crocks of sterilized and 5 of unsterilized soil were used in each test.

Table 7 shows that the soil first inoculated with *Pythium ultimum* reacted in a manner directly opposite to that of the soil first inoculated with *Rhizoctonia solani*. For example, the greater preemergence loss for the *P. ultimum* series was in the sterilized inoculated soil, whereas that for the *R. solani* series was in the unsterilized inoculated soil. Conversely, the heaviest postemergence damping-off occurred on the unsterilized soil when it was first inoculated with *P. ultimum*, and the heaviest loss occurred on the sterilized soil when it was first inoculated with *R. solani*. This apparent contradiction is clarified by the isolation data presented in table 8.

TABLE 8.—*Isolates obtained from Siberian elm seedlings showing postemergence damping-off symptoms in test presented in table 7*

Isolate	Isolations from—									
	Pythium-inoculated soil plus *R. solani* 5 days later				*Rhizoctonia*-inoculated soil plus *P. ultimum* 5 days later				Uninoculated unsterilized soil	
	Sterilized soil		Unsterilized soil		Sterilized soil		Unsterilized soil			
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Pythium ultimum	3	60	3	7	2	4	0	.....	5	28
Rhizoctonia solani	1	20	27	59	42	82	8	62	1	5
Trichoderma sp	1	20	0	.....	1	2	0	.....	0	.....
Fusarium sp	0	.....	5	11	1	2	1	8	8	45
Phycomycetes	0	.....	2	4	3	6	0	.....	1	5
Alternaria sp	0	.....	0	.....	2	4	0	.....	1	5
Aspergillus sp	0	.....	1	2	0	.....	1	8	0	.....
Penicillium sp	0	.....	0	.....	0	.....	1	8	1	5
Bacteria	0	.....	6	13	0	.....	2	15	1	5
Unclassified	0	.....	2	4	0	.....	0	.....	0	.....
Total	5	100	46	100	51	100	13	101	18	98

All these tests show that both *Pythium ultimum* and *Rhizoctonia solani* are definitely parasitic on broadleaf seedlings. Pathogenicity can be obtained in either sterilized or unsterilized soil, depending upon whether the tests are for preemergence or postemergence damping-off. Preemergence losses caused by *P. ultimum* apparently can be better demonstrated for broadleaf species by inoculation in sterilized rather than unsterilized soil, but *R. solani* inoculations were more effective in unsterilized soil. On the other hand, for producing postemergence damping-off, these tests showed that the percentage loss was greater when *P. ultimum* was inoculated into unsterilized soil and *R. solani* into sterilized soil.

DISCUSSION

The information presented in this paper is believed to be fundamental to a complete understanding of the damping-off problem as it affects seedlings of broadleaf species in Great Plains nurseries. Because of the uniformity in climatic conditions and soil formations through the Great Plains region, control measures that are successful in Texas are likely to be equally so in North Dakota. In some respects the problem of controlling damping-off of broadleaf species is similar to that of its control in other crops because the principal parasites in both cases are *Pythium ultimum* and *Rhizoctonia solani* and on occasion *Fusarium* spp. Since the seedlings are commonly grown in open drills and not in protected seedbeds, successfull large-scale control measures will necessarily be quite different from those used in coniferous nurseries.

Only about half of the broadleaf species studied proved to be susceptible to damping-off; therefore the control problem at once becomes considerably simplified. Since some soils of the region showed heavier damping-off potentialities than others, only those broadleaf species resistant to damping-off should be grown on unfavorable sites. For susceptible broadleaf species, control methods should take into consideration the fact that both *Pythium ultimum* and *Rhizoctonia solani* are pathogenic and that *Fusarium* is apparently also capable of causing postemergence losses when temperature conditions are favorable. Treatments that inhibit one of these parasites may encourage the development of another. Moreover, there does not appear to be a marked difference in virulence between *Rhizoctonia* strains; hence, broadleaf species susceptible to damping-off should not be grown in the area occupied during the preceding season by other susceptible species. If these findings are taken into consideration, control measures should be somewhat simplified and more effective.

SUMMARY

Soil developmental processes and climatic conditions in the Great Plains are unusually uniform for a region extending through so many degrees of latitude.

Approximately 50 percent of the broadleaf species grown are susceptible to damping-off, four of the most important being the American elm, Siberian elm, black locust, and desertwillow.

For susceptible species, damping-off mainly assumes two forms. When the seed or new radicle is destroyed, the losses are classified as

preemergence damping-off. Postemergence damping-off results from infection of seedlings from a few days to 3 weeks old, and the diseased plants may either fall prostrate on the soil or, more commonly, remain erect, dry up, and break off. Sore shin is the result of stem infection at about ground level of older plants. Root rots and top infection of young seedlings are unimportant in this region.

The principal damping-off fungi have been identified as *Rhizoctonia solani* Kühn and *Pythium ultimum* Trow; *Fusarium* spp. may also be of occasional importance. *R. solani* isolates from different broadleaf species are regarded as strains, since cultural characteristics were not constant enough, in the opinion of the writer, to justify the establishment of species. Of the broadleaf species tested, black locust appeared to be the most susceptible to *P. ultimum* and desertwillow the most susceptible to *R. solani*.

Pathogenicity tests have proved that *Pythium ultimum* and several strains of *Rhizoctonia solani* are definitely pathogenic to a number of broadleaf species and also to several kinds of agronomic plants. *R. solani* inoculations appeared to be more successful in unsterilized soil than in sterilized soil, whereas the opposite was true for *P. ultimum*. The comparatively wide range of pathogenicity of *R. solani* isolates further justified designating them as strains rather than species. Preliminary tests indicated that *Fusarium* spp. can cause fairly heavy postemergence damping-off in unsterilized, inoculated soil but very little preemergence loss except in sterilized, inoculated soil.

The relation of these studies to the control of damping-off of broadleaf species is discussed.

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